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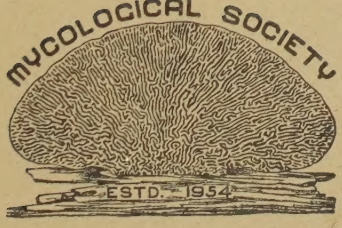

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INDIAN JOURNAL

OF

MYCOLOGICAL RESEARCH

(Official Organ of Mycological Society)

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FOREWORD

WHENEVER a new Society is started, the question is rightly raised "Why ?". It must be admitted that Fungi are a remarkably successful group, rivalling flowering plants in number of species and vastly outnumbering them in individuals. There can be no question that a serious student of modern Botany should have a general idea of the fungus flora in his neighbourhood. Popular ignorance of this group of plants is little short of amazing. At the present time in India fungi are looked upon as something abnormal and worthless. They are only made to be destroyed. No eye can appreciate their beauties, they are hardly allowed a lawful place among Nature's children. This state of affairs should be combated by establishment of regular field clubs or foray-societies which will create interest amongst the public by holding periodical exhibitions of local fungal flora freshly collected. With this object in view, the Society arranged a number of Sunday excursions during the entire rainy season of 1955. If the collection of fungi can be systematically carried on by our enthusiastic younger members from year to year for for more than a decade, we are sure to accumulate many interesting data. In any scientific undertaking plodding and persistent work is of sterling value. Periodicity in appearance is a problem which can be solved only by field studies for successive years and succeeding seasons. Is there a sequence in appearance and a curve of abundance according to meteorological conditions ?

So far as higher fungi (*Polyporaceae*) are concerned, systematic surveys in Bengal and special studies of some groups have been carried out. Of course, much remains to be done in the way of study of anatomy and of functional anatomy. Some of the Polyporists of the world, whose number barely exceeds a dozen, are now turning their serious attention to this study. It is a matter of gratification that important works in India on *Fusarium*, Rusts, Smuts, *Synchytrium* and *Cercospora* are going

on in right earnest. It is hoped this may be a source of stimulation to workers in different areas of India so that similar studies should be gradually extended to important groups of macro- and micro-fungi.

I wish 'Bon voyage' to this new Society's Journal and hope and pray that it will grow from strength to strength every year.

"When Flora's lovelier tribes give place,
The Mushrooms' scorn'd but curious race
Bestud the autumnal earth ;
A quick but perishable birth,
Inlaid with many a brilliant die
Of Nature's high—wrought tapestry."

S. R. Bose

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A DISEASE OF NORWAY SPRUCE (*PICEA EXCELSA* (LAM.) LINK.) ASSOCIATED WITH *STEREUM SANGUINOLENTUM* (A. et S.) FR. AND *PLEUROTUS MITIS* (PERS.) BERK.

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(With Plates I to VIII and 1 Text-figure)

Abstract

This study has been undertaken to provide information which would assist in evaluating the importance of a serious disease of Norway spruce growing in a mixed stand in Glentress forest, near Peebles, in Scotland. Two fungi, viz., *Stereum sanguinolentum* and *Pleurotus mitis* have been found to be the cause of the disease. They have been isolated from the internal rots of the same or different standing trees. Of these, *S. sanguinolentum* appeared more commonly during isolation.

The external and internal symptoms of the disease have been fully described, the essential features being pronounced fluting of the stem as a result of longitudinal depressions in the bark and its subsequent cracking with copious outflow of resin. The infected lesions are mostly restricted within the limits of pruning and occur round about the pruning wounds where strips of cambium have been killed due to the entry of the pathogens.

The isolates of *S. sanguinolentum* and *P. mitis* have been grown in culture. Their characteristic appearance and constant behaviour have been presented in potato-dextrose agar and malt agar. On these two media both the fungi behave very similarly as a rule. In all isolations of each fungus the cultures have started in the same way but the growth rates have been greater on potato-dextrose agar than on malt agar. They are both 'white rot' fungi since they show positive oxidation reactions.

Typical fructifications of *S. sanguinolentum* and *P. mitis* bearing normal basidia and basidiospores have been obtained from mycelia originating from the infected tissues of Norway spruce. Various devices adopted to induce the cultures to fructify have been fully described. A low range of temperature (5°C–15°C) has been found to be essential for the production of sporophores of *P. mitis*, below or above which fruit-bodies are not formed at all. *S. sanguinolentum*, on the other hand, can fructify well under ordinary conditions of light and temperature of the laboratory.

Fructifications of both *S. sanguinolentum* and *P. mitis*, that developed on sections of infected trees after felling, have been thoroughly described and their anatomical peculiarities noted.

Microscopic characters of the rots have been fully described. The rotted tissues show the presence of mycelium in all stages of decay, appearing most abundantly in the advanced stages. It is particularly plentiful in the medullary rays which seem to be the first element to be attacked. Closely interwoven mycelium often fills up many of the ray parenchyma cells, sometimes tracheids and associated with gum-like material giving the appearance of a zone-line. The mycelium ramified at first through the pits but later penetrates the cell-walls forming numerous bore-holes.

Chemical changes of the wood during the process of decay have been tested with micro-chemical stains in common use. The change consists mainly in the delignification of the highly lignified parts of the secondary walls of the wood-elements associated with digestion of cellulosic materials. It is also evident

that in the early stages of decay *S. sanguinolentum* can utilize more lignin than *P. mitis* while the latter digests more cellulose at the beginning.

The natural resistance to decay of spruce timber has been tested in the laboratory by exposing small sterilized test-pieces of the sound timber to the attack of *S. sanguinolentum* and *P. mitis* separately growing in pure culture under controlled conditions, and the amount of decay resulting from the fungal attack after 4 and 8 months' tests has been calculated by estimating the loss in dry weight. After the experiments it has been found that the spruce timber is 'non-resistant' to decay. It has been found that the loss in weight due to *P. mitis* is less than that caused by *S. sanguinolentum* both after 4 and 8 months' studies. But the interesting fact is that the loss in weight after 8 months is double of that after 4 months in both cases.

Inoculations have been made on cut twigs in the laboratory and also on trees in the field. It has been observed that when freshly cut surfaces of these twigs are inoculated with spore-suspensions of *S. sanguinolentum* and *P. mitis* in sterile water, the spores germinate readily on the surface within a short time and send down hyphae into the elements of the wood. Since these twigs cannot be kept in an actively growing condition, their invasion by *S. sanguinolentum* and *P. mitis* cannot be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores some how alight on the surface of the wound, they can germinate there under suitable conditions and pass down their hyphae into the elements of the wood. On the other hand, inoculation experiments on living trees carried out in the field have shown that both *S. sanguinolentum* and *P. mitis* can infect living trees through wounds in the bark, and from the results of inoculations they can be regarded as wound parasites on normal vigorous trees.

Introduction

It has been observed that about 20 per cent of forty to sixty years old Norway spruce trees (*Picea excelsa* (Lam.) Link.) growing in a dense, mixed, stand with Sitka spruce and Douglas fir at Glentress forest near Peebles in Scotland, have been for the last four or five years in a diseased condition and are gradually dying. The trees are badly shaped and in most cases there is considerable amount of resin flow from the bark on the lower trunk. The trees do not give any evidence of the presence of frost rings or cracks in their wood. This plantation occupies a hill-slope of moderate gradient, leading to the top of the hill (about 700–1000 feet above sea-level). On the whole it is a cool, damp and muddy situation and areas of bad drainage occur here and there. Since the economic value of Norway spruce as a timber-yielding plant is well known, it is thought that the information concerning the causal agent, and the amount of damage caused by it is desirable. With this end in view, the present investigation has been undertaken during the latter half of 1949. It is interesting to note here that during this investigation two fungi, viz., *Stereum sanguinolentum* and *Pleurotus mitis* have been found to be associated with the disease and these are capable of infecting living trees through wounds, eventually causing rot in the wood. It is apparent that this association of two pathogens has greatly enhanced the importance and complex nature of the problem. At the present moment it is difficult to estimate the relative importance of the two fungi in bringing about the decay in living trees and the nature of reactions existing between them. An investigation of this nature would necessarily require a longer period owing to the slow growth of the fungi and their effects on a living host. The data, so far obtained, have been given in the following pages based largely on studies in the laboratory as well as in the field.

Historical

Gäumann and Jaag (1937) have described a disease in spruce and fir causing severe damage in a re-afforestation area of the Emmentel in Switzerland. The symptoms described by them are somewhat similar to those observed in the present study and are attributed only to the virulent pathogen. *Pleurotus mitis*. Although this fungus has been known to cause heart-rot of conifers on the continent of Europe, yet up to the present it does not appear to have been recorded on living trees in Great Britain.

Recently, Day (1950) has reported a severe case of bark necrosis on Norway spruce in Drummond Hill Forest in Perthshire, Scotland. The injuries are diagnosed by him as having been caused by frost damage usually after pruning live branches. He has reported and figured cracking of the bark with consequent outflow of resin. The effect of the injuries on the wood of the tree, however, has not been fully investigated by him. He mentions that under such conditions some degree of fungal infection is inevitable although it has not yet been possible for him to say what types of fungi may have entered.

The same type of injury has been reported by Laing (1947) on Sitka spruce and the cause of such damage has been attributed to *Nectria cucurbitula*. He has found abundant mycelium and conidia of *N. cucurbitula* on the dead bark but he did neither actually isolate the fungus from the internal tissues nor carried out any infection experiments to prove its pathogenicity.

Although *Stereum sanguinolentum* has long been known to occur commonly on dead stumps and branches of coniferous trees in Great Britain and on the continents of Europe and America, there still exist contradictory statements in the description of the rot made by various workers from time to time. As such it will be evident that the external symptoms exhibited by the infected trees under consideration are somewhat different from those described by previous workers. In Great Britain very little is known about its parasitic activity although it is reported to cause a butt-rot of coniferous trees (Day and Peace, 1936) and timber-stain in Norway spruce (Cartwright, 1937). Cartwright and Findlay (1946) have isolated it from freshly felled poles of spruce and larch and incidentally referred to its sporadic occurrence in living trees. In the continent of Europe it occurs on various conifers in Denmark (Ferdinandsen and Jorgensen, 1938–39) and Germany (Bergenthal, 1933) and also as a wound-parasite in fir and pine (Rohmeder, 1937). In Russia, it causes a slow decay in fir and pine (Vakine, 1934). This fungus is also known to attack living spruce in Norway (Jorstard and Juul, 1939) and in Sweden (Lagerberg, 1919, 1923) where it gains entrance through top-breakage due to snow damage or through wounds. Robak (1936) has isolated it from heart-rot of living spruce and mentioned (Robak, 1942) that it is commonly found on dead coniferous wood in Sweden. In America, it causes a heart-rot of balsam fir (Faull and Mounce, 1924; McCallum, 1925, 1928), often causing a top-rot (Kaufert, 1935). It also induces a sap-rot causing much damage in firs, Douglas firs, larches, pines and spruces (Hubert, 1935; Baxter 1937).

It is a dangerous fungus in pruning wounds of northern white 'pine (Spaulding and Coll., 1935) and the principal agent causing 'slash' decay (Spaulding, 1929). Thus, it becomes event that the importance of the fungus has been gradually recognised although it is difficult at the present moment to estimate the amount of losses caused by this fungus. The indications are such that it may prove to be a serious pathogen in Great Britain and the losses caused by it may ultimately become very serious.

Symptoms

The symptoms of the disease are few and conspicuous. The infected trees show longitudinal depressions of the bark (Pl. I, figs. 1 & 2) which may often be ill-defined and somewhat flat. These extend from the butt upwards up to 6 feet, but occasionally up to a height of 10 -12, feet, giving the trunk a somewhat fluted appearance. The bark of the sunken areas is often discoloured. Its underlying tissues die, become dessicated and eventually it splits lengthwise accompanied by copious outflow of resin (Pl. III, fig. 7). Gradually, these areas appear to sink deeply into the trunk owing to continued growth in thickness in the areas lying between them. The lesions usually occur round about pruned branches, run up and down the trunk and are restricted within the limits of pruning. They may sometimes extend towards the base of the trunk where fluting becomes most pronounced (Pl. I, fig. 2). The bark near the base of the trunk ultimately becomes loose and separates easily, particularly when the poles are cut and dried. A mass of whitish discoloured tissue and mycelium is often present in between the bark and the sapwood. The inner surface of the bark and the surface of the sapwood in contact with it show a white mottled appearance.

Cross sections of freshly felled poles in the early stages of decay show irregular to wedge-shaped areas (Pl. III, figs. 1, 2 & 4) at the periphery. These are light to dark brown, sometimes reddish-brown in colour. They gradually extend towards the centre of the wood into the base of the trunk and finally coalesce causing a butt rot (Pl. III, fig. 3). In such cases the course of infection appears to be from the bark to the centre of the stem. Close examination of the infected area reveals that the cambium is killed near the pruned branches. This is possibly due to the entry of the pathogens through dead branches or other injuries such as pruning cuts and after killing the cambium they spread from the periphery to the centre of the wood. The organisms seem to spread much more rapidly in a longitudinal direction than laterally. In this way, longitudinal strips of cambium are killed near the pruned branches and as a result further secondary growth cannot continue. In the areas between these dead cambial strips growth continues and the trunk ultimately shows prominent ridges. The transition from the normal to decayed wood is abrupt and there is no sign of any black line of decay. The discoloured areas, in an advanced stage, show small pockets which are somewhat whitish and hollow.

There was no sign of any fructification of the causal organisms on the surface of the infected trees but recently felled logs of such trees that had

lain on the forest floor were found with fructifications of *Stereum sanguinolentum* and *Pleurotus mitis* over the rotted areas on the surface of the cross sections (Pl. I, figs. 3 and Pl. II, fig. 5).

The causal agents

Materials for study

The materials were obtained in November, 1949, in the form of freshly felled infected trees from Glentress forest near Peebles in Scotland. The fructifications of *Stereum sanguinolentum* and *Pleurotus mitis* were also collected while growing in an excellent condition on the surface of the cross sections of recently felled Norway spruce poles lying on the floor of the forest near the infected trees. The diseased trees were carefully selected, felled and macroscopically examined in the field to note, as far as possible, the progress of decay in the wood. For further study, small cylindrical sections from different regions of the infected trunk were selected, brought into the laboratory, examined and studied while in a fresh condition. Microscopic examination of the rotted areas revealed the presence of abundant mycelium within the tissues of the host.

Isolation and identification of the causal organisms

The mycelium from the host tissues was isolated and studied. The isolation was made from the infected wood by using portions of isolated rotted tissues from selected regions of the same or different poles. Small pieces (about $2'' \times \frac{1}{2}'' \times \frac{1}{2}''$) of such wood blocks were washed several times with sterile distilled water, dipped quickly in rectified spirit and flamed in order to free the surface, as far as possible, from superficial contaminations. One side of the exterior portion of such a wood block was removed with a sterile scalpel. A small piece of wood was then carefully cut out from the freshly exposed surface near the edge of the decayed area and transferred aseptically to a tube containing 2% malt agar slant into which it was partially embedded. In this way several transfers were made. Cultures were also made from poles bearing fructification of *Stereum sanguinolentum* and *Pleurotus mitis* on their cross-section-ends. These were then incubated at 23°C. in darkness. In most cases the mycelia grew out within a week and sub-cultures were made from each of these in 2% malt agar tubes which were subsequently used as stock cultures. After close examination of all the isolates it was found that these cultures belonged to two different fungi and exhibited the characteristics of the cultures of *Stereum sanguinolentum* and *Pleurotus mitis*. These were compared with the polysporous cultures of *Stereum sanguinolentum* and *Pleurotus mitis* already made available for the purpose and found to be identical with the latter in both cultural and microscopic details. The identity of these isolates was further confirmed when these cultures subsequently produced typical fruiting bodies in artificial cultures (Pl. II, fig. 6 and Pl. IV, figs. 1 & 2).

Spore cultures of *Stereum sanguinolentum* and *Pleurotus mitis* were also made in the usual way. The spores germinated readily on 2% malt

agar slants within 12 to 24 hours and good mycelial growth was obtained in all the tubes within a fortnight. These were used as stock cultures for further study and comparison.

In a great majority of cases the isolates were those of *Stereum sanguinolentum* but nevertheless *Pleurotus mitis* was also isolated from separate regions of the same or different poles. In one instance both the organisms were not only isolated from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. This statement was further corroborated by inducing the fungi to produce fructifications on the rotted areas at the cross-section-end of the infected pole from which isolations were made (Pl. III, fig. 6). From the number of isolations so far made, it was evident that *Stereum sanguinolentum* was more widely distributed within the host tissues than *Pleurotus mitis*. Further investigations in future with isolations made from large numbers of infected trees would possibly throw some light as to their relative distribution within the host plant.

The sporophores

1. *STEREUM SANGUINOLENTUM* (A. et S.) Fr. (Plate I, figs. 3 & 4).

Fructifications. Sessile, at first forming somewhat orbicular and resupinate patches, then narrowly reflexted, later becoming laterally confluent and forming broad continuous sheet, measuring 30—40 × 30—50 m.m. or more; reflexed portions about 2 m.m. deep from margin to back, often becoming densely imbricated and laterally confluent, somewhat folded: dimension of the resupinate position usually 10—15 m.m. but in younger specimens much less (2–3 × 3–5 m.m.); thin and leathery; margin thin, acute, usually white in colour. *Upper surface.* Villose; with somewhat satiny and radiately adpressed hairs; faintly zoned near the margin; pinkish buff* or pale olive buff to pale drab gray or pale smoke gray towards the margin. *Context.* Thin; coriaceous; whitish. *Hymenial surface.* Smooth, often with small isolated tubercles; slightly wrinkled at places; becoming irregularly cracked in the resupinate portion on drying; bleeding red when cut or bruised; pale olive buff, olive buff or fuscous, becoming avellaneous or wood brown on drying. *Basidia.* Cylindrical to clavate; dimension about 30–40(50) × 4–8μ; di- to tetrasterigmatic; sterigmata 4–5μ long (text-fig. 1, a). *Spores.* White in mass, otherwise hyaline; smooth; oblong or slightly curved; with an apiculus; dimension about 6–7.5(8.5) × 2–3μ (text-fig. 1, b). *Lactiferous cells.* Numerous; reddish-brown in colour; arising from the layer below the hymenium and extending into it; elongated but clavate near the apex; about 300–400 × 4–5μ but near the apex 5–7.5μ wide; bleeding when broken; when mounted in glycerine the contents break up into somewhat rectangular blocks. *Tissue differentiation.* Entire fructification about 300–400 (600)μ thick and the following regions can be differentiated : (*a*) a hymenial layer up to about 50μ thick; (*b*) an intermediate hyphal layer measuring 150–250μ thick,

* Ridgeway (1912).

composed of densely arranged hayline hyphae and from this layer coloured conducting organs originate and curve upwards into the hymenium; (*c*) a narrow golden yellow zone, about 50μ wide lying between the intermediate layer and the outermost hairy covering, composed of longitudinally arranged hyphae: and (*d*) the outermost hairy covering composed of densely matted hyphae, about 50–100 (200)μ thick. *Hyphal characteristics.* Two kinds of hyphae can be recognised : (1) thick-walled, matted or loosely interwoven hyaline hyphae of the hairy covering, more or less straight, sometimes slightly flexuous, mostly unbranched, distantly septate, lumen often almost obliterated, without clamp-connections, about 2.5—5(7.5)μ wide and sometimes with a few granular contents, and (2) comparatively thin-walled interwoven or longitudinally arranged hyphae of the intermediate layer and golden yellow zone, sparingly branched, distantly septate, without clamp-connections, sometimes pale yellow in colour, with granular contents, about 3—5μ wide.

The fructifications of this fungus develop readily on freshly felled poles of infected trees during the months of October to January. They are found either on the cross-section-ends over the rotted areas or on the cracked bark. They are commonly recognised in having thin, coriaceous and resupinate fruiting bodies and with a narrowly turned back margin. The hymenium, somewhat drab in colour, bleeds red when wounded and cracks irregularly and deeply on drying. Numerous coloured lactiferous cells in the hymenium and underlying tissues are conspicuous diagnostic characters by which the species can be readily identified. Owing to their small size they are difficult to recognise from a distance and as such, of little help as an indication of decay in standing trees in the forest. According to Burt (1920) and Rea (1922) there are no cystidia but recently Wakefield and Dennis (1950) have mentioned the presence of yellowish brown cystidia in the hymenium. They have however, not stated anything about the presence of numerous lactiferous cells so characteristic of the species and as such yellowish brown cystidia described by them are in reality the conducting organs of the previous workers. These organs bleed i.e., their contents come out when broken and this is the unique character of the conducting organs and not of cystidia as given by Overholts (1929).

2. *PLEUROTUS MITIS* (Pers.) Berk. (Plate II, figs. 1–3)

Pileus. Sub-sessile or shortly stipitate, continuous with the stipe in a more or less horizontal plane; thin, slightly fleshy, tough and fexible; sub-spathulate to somewhat reniform; about 5–15 (20) m.m. across. *Upper surface.* At first entirely white, later becoming slightly rufous or showing shades of salmon colour or flesh ochre near about the stipe; smooth, glabrous, often showing fine longitudinal striations near the margin; margin entire, involute when young or on drying. *Flesh.* Thin; sub-gelatinous below the cuticle; white; less than .5 m.m. thick. *Gills.* Very narrow, linear-lanceolate; crowded, adnate; white, becoming pale salmon colour with age or on drying; about 1–1.5 m.m. wide. *Stipe.* Short, lateral, widening into the cap; white or tinged rufous; somewhat compressed; covered all over with whitish, mealy squamules; about 1–3 m.m. long,

sometimes becoming much elongated (6–12–15 m.m.) when developed within cracks, about 1–1.5 m.m. thick. *Basidia.* More elongated than the immature ones; narrow clavate; with 2–4 strigmata (1.5–2μ long); dimension about 16–20–25$\times$3.5–4μ; immature basidia about 9.5–12.5$\times$ 3–3.5μ (text-fig. 1, i). *Spores.* Sausage-shaped; hyaline; smooth; dimension about 4–5$\times$1.5–2–2.5μ (text-fig. 1, h). *Tissue differentiation.* A vertical section through the upper fleshy portion of the pileus shows three distinct regions : (*a*) the outermost brownish cuticular layer, about 20–30μ thick, (*b*) an intermediate sub-gelatinous layer, about 180–200μ thick, with loose stainable hyphae embedded in a gelatinous matrix, and (*c*) a more or less brownish basal portion, about 80–100μ thick. In a cross section of the stipe three different regions can be distinguished, viz., (*a*) a dark compact and circular central zone, about 900–1200μ thick and consisting of densely interwoven hyphae, (*b*) an intermediate hyaline sub-gelatinous zone, about 200–300μ thick and with loosely interwoven hyphae and (*c*) the outer most compact zone of about 150–200μ in thickness. A transverse section of the gill shows a central sub-gelatinous trama, about 60-150μ wide, composed of compact parallel hyphae and its central axis loosening from the sub-hymenium and hymenium. *Hyphal characteristics.* Two kinds of hyphae appear to have entered into the composition of the fruit-body viz., (*i*) thin-walled, hyaline, wider hyphae, straight and more or less parallel, sometimes slightly flexuous, distantly branched and septate, with a few granular contents, sometimes empty, about 2.5–4(5)μ wide, without clamp-connections and with walls often partially gelatinised, and (*ii*) thin-walled, hyaline, narrow hyphae, about 1.5 to 2.5μ wide, flexuous, sparingly branched, with clamp-connections, full of granular contents and more or less intertwined.

The afore-said two kinds of hyphae are characteristic of the gills and the stipe. In the gill the wider hyphae are more numerous than the other type and appear to run more or less parallel within the trama. The narrower hyphae, on the other hand, are highly flexuous and embedded in a gelatinous matrix within the stipe. The fleshy portion of the pileus is entirely made up of this type of narrow fluxuous hyphae similarly embedded in a gelatinous matrix, the wider hyphae being unrecognisable as a result of gelatinisation.

This fungus, long called *Agaricus mitis* Pers., is well known in France, Switzerland as well as in all of Europe and is frequently found on dead twigs and stumps of coniferous trees. Berkeley (1860) mentions that it occurs on larch in Scotland and is abundantly found in Nottinghamshire. The writer has collected it growing on dead twigs and stumps of Norway spruce, Sitka spruce and Douglas fir from Peebles, Scotland. It has also been collected while growing saprophytically on Scots pine in this country. Josserand and Smith (1937) state that this species is identical with the American species *Panus bacillispora* Kauffman and suggest that these two names should fall into synonymy with *Panellus mitis* (Pers.) Singer. In order to avoid confusion, the writer has retained the older name *Pleurotus mitis* by which it is familiarly known in Great Britain and on the continent of Europe. *Pleurotus mitis* is easily recognised by its tiny, white fructifications which later become somewhat refuscent. Though not externally

recognised, its gelatinous flesh and trama and narrowly allantoid spores are distinctive characters. The fructiacations revive after a period of dessication and are gregarious.

Fungi in culture

The isolates of *S. sanguinolentum* and *P. mitis* from Norway spruce were grown in culture and their characteristics studied on two different media, viz., *potato-dextrose agar* and *malt agar*. Potato-dextrose agar was prepared according to the recipe of Fritz (1923). All cultures were kept in darkness in different incubators at 18°C. and 23°C. In order to maintain experimental conditions as constant as possible, small circular discs of inocula (about 3-4 m.m. in diameter) were taken from young cultures and placed with upside downwards upon the media in tubes and petri-dishes, each containing approximately 20 c.c. and 30 c.c. of the medium respectively. The pH value of each medium was adjusted and after sterilization was found to be 5.2. The colour exhibited by the mycelium has been expressed according to Ridgeway (1912) and the terms used to describe the texture of the mat are those proposed by Long and Harsch (1918) and Nobles (1948).

In culture *S. sanguinolentum* was studied repeatedly by various workers such as Lagerberg (1924), Bavendamm (1928), Bergenthal (1933), Cartwright and Findlay (1938) and others but their descriptions were widely divergent. Robak (1942) made an extensive study of the different strains of the fungus and divided them into three main types. It has been found that the present isolate does not strictly fall entirely to any of the groups as given by Robak (1942). Very little is known about the detailed cultural characteristics of *P. mitis* although Gaumann and Jaag (1937) determined the maximum, optimum and minimum temperatures for the development of the fungus on malt agar.

Oxidase tests

There are evidences in support that the intensity of attack on lignin is directly dependent upon the oxidase activity of the white rot fungi and this can be detected by the method described by Bavandamm (1928). This oxidase test was made by growing the fungi in Petri dishes on 2.5% malt agar containing 0.5 per cent of gallic or tannic acid and kept at a constant temperature of 23°C. in darkness. Dark brown rings appeared within 24 hours around the inocula of both the fungi indicating the presence of oxidase. This positive reaction indicate that both the isolates of *S. sanguinolentum* and *P. mitis* were white rot fungi but their intensity of reaction showed some variation. On gallic acid medium within 24 hours after inoculation *P. mitis* showed very intense reaction and the diameter of the ring was 20–25 m.m., whereas the diameter of the same produced by *S. sanguinolentum* was only 10 m.m. and the reaction was less intense than the other. On tannic acid medium the reactions were intense to very intense in both cases. Another method to distinguish white rot fungi from brown rot ones (Preston and McLennan, 1948) was successfully

tried by growing both the fungi on 2.5% malt agar containing 0.007 per cent gentian violet. Being white rot fungi, the violet colour of the medium was bleached slowly by them but about 6 to 8 weeks incubation was necessary before positive results could be obtained. According to Preston and McLennan (1948) complete decolorization of the dye would take place only when the oxygen concentration above the medium was greater than 6% and this discolouration was probably due to the production by the white rot fungi, of an extracellular oxidase system.

Cultural characteristics

A. *STEREUM SANGUINOLENTUM* (A. et S.) Fr.

(*i*) Habit of growth (Pl. IV, figs. 3, 5 & 6). On *potato-dextrose agar* at 18°C. the growth of the mycelium started with moderate rapidity. It was initially thin and arachnoid with an even advancing zone. During the first seven days after inoculation it remained in this condition excepting on the transplant where a shaggy ball was most often present. As the growth advanced, the mat proper, at first glabrous, gradually became denser and covered the whole surface of the slant with soft, downy-woolly mycelium, somewhat thin, appressed and fringed at the margin. In about 12 days after inoculation the mycelium tended to fill the base of the tube with a compact felty mat which in the older part, was depressed and somewhat opaque. The new growth was downy to cottony-woolly and somewhat transluscent, particularly towards the upper end of the tube. The colour of the medium changed to yellowish with a pinkish tinge. Yellowish glistening drops of liquid appeared over the inoculum and on the mat in about three weeks time but later these disappeared. At 23°C. the growth was more or less the same but the condensation of the mat seemed to be more pronounced. On *malt agar* under identical conditions the growth was rather slow, thin and arachnoid at the beginning but somewhat cottony to felty over the inoculum and not a shaggy ball. As the growth advanced the mat became felty, patchy and in about a month, formed a tough skin-like mat, difficult to tear. In general, growth and subsequent condensation of the mycelium to form a thick mat was less pronounced in malt agar than that in potato-dextrose agar.

(*ii*) Colour. On *potato-dextrose agar* at 18°C. colouration appeared first after a week's growth over the inoculum as a patch of apricot buff which later deepened to buckthorn brown or mummy brown. Within a fortnight after inoculation, pigmentation of the mat at 23°C. was confined to the area just around the inoculum and included shades of antimony yellow, buckthorn brown and mummy brown, while the major portion of the mat remained white. Within a week after this, diffused antimony yellow colour appeared over the whole mat but it was more pronounced towards the lower end of the slant which later deepened to patches of ochraceous orange. Although pigmentation started early at 23°C., later it was more pronounced in cultures kept at 18°C. On *malt agar* at 18°C. colouration of the inoculum and the surrounding mat started in about 12 days after inoculation and was pinkish buff in colour. Later

Text-fig. 1. Basidia, basidiopores and aerial and submerged hyphae of *S. sanguinolentum* (*a–g*) and *P. mitis* (*h–l*) (*c–g*, *j–l*, × 650). See text.

other shades such as warm buff, antimony yellow and ochraceous orange developed on the mat. These were restricted to limited areas of depressed mycelium of a denser texture. At 18°C. colouration of the mat seemed to be more pronounced than that at 23°C. and also more pronounced than that at 18°C. and 23°C. on potato dextrose agar.

(*iii*) Rate of growth. The increment in diameter of Petri dish cultures on 2.5% *malt agar* at 23°C. in darkness were determined as follows :—

Days	2	4	6	8	10	12
Average diameter of growth (m.m.).	15	30	45	60	75	90

These figures indicate that the fungus grows with moderate rapidity, with an average daily increment in diameter of 15 m.m. At 18°C. on the same medium the growth was slightly slower with an average daily increment of growth of 14 m.m. On *potato-dextrose agar*, on the other hand, the rate of growth at 23°C. was much more rapid than that on malt agar with an average daily increment in diameter of 17 m.m.

(*iv*) Hyphal characters (text-fig. 1, c–g; Pl. V, figs. 1, 2 & 4).

Advancing zone. Hyphae hyaline, straight or flexuous, sparingly branched, usually distantly septate, with frequent large, simple clamp-connections, sometimes in opposite pairs or whorled, about 3–6(7)μ wide, with conspicuous granular contents, sometimes pale yellow in colour. *Aerial mycelium.* Three types of hyphae can be recognised, viz., (1) wider thin-walled hyphae, more or less straight or slightly flexuous, usually distantly septate, with a few simple or paired, arched clamp-connections, very sparingly branched, usually 5.5–6.5μ wide but may be up to 7.5–8μ broad, sometimes with yellowish-brown contents, often giving rise to finer hyphae from the sides which may be clustered at the septa (text-fig. 1, c–g; Pl. V, figs. 2 & 4); (2) thin-walled narrower hyphae, springly branched, distantly septate, rarely with simple clamp-connections which may grow out to form new hyphae, about 2.5–4.5μ wide, often spirally coiled (Pl. V, fig. 1), with or without granular contents, hyaline or brownish in colour (text-fig. 1, c, e); (3) very narrow, thin-walled, hyaline, much branched hyphae originating from the wider or intermediate types, often flexuous, with dense, granular contents, without clamp-connections (text-fig. 1, f). *Submerged mycelium.* The same three types of hyphae can be recognised but with slight differences as follows—(1) wider hyphae very numerous, hyaline, mostly with granular contents, about 5.5–6.5μ wide, often slightly constricted at the septa, without clamp-connections; (2) intermediate type fairly numerous, sparingly branched, branches flexuous, with or without granular contents, hyaline or faintly coloured, distantly septate, rarely spirally coiled; (3) finer hyphae very few. Crystals large, numerous.

B. *PLEUROTUS MITIS* (Pers.) Berk.

(*i*) Habit of growth (Pl. IV, figs. 4 & 7). On *potato-dextrose agar* at 18°C. (Pl. IV, fig. 3a) the growth was at first slow being only confined to the inoculum as a dense, white and felty covering. As the growth

advanced, the mycelium condensed over it forming a felty ball while on the agar surface it remained entirely appressed, sodden and colourless with indistinctly fimbriated margin. In about 10 days after inoculation, the mat proper became differentiated into a broad central, white and felty zone around the inoculum and a distinct appressed and colourless advancing zone, about 2–3 m.m. wide. In cultures about 20-days-old the mat presented the appearance of a dense, white and smooth felt while the region just behind the advancing zone tended to become somewhat raised and sub-felty. In old cultures both the central and advancing zones became felty but were separated from each other by a narrow sub-felty area and eventually a thin skin was formed. Considerable discolouration of the medium was noted in cultures 10-days-old and it was pale yellow. At 23°C. (Pl. IV, fig. 3b) on the other hand, the habit of growth was more or less the same but the rate of growth was rather slow and the mat was more compact. On *malt agar*, both at 18°C. and 23°C. (Pl. IV, fig. 3c, d) the growth was poor and the mat proper remained mostly appressed and colourless for a considerable length of time. Towards the end of the second week after inoculation a felty to pulverulent central zone was differentiated and an outer appressed and colourless zone of variable width (2–8 m.m.) could be clearly recognised.

(*ii*) Colour. On both the media all cultures remained white throughout and no other pigment developed even in cultures 2 to 3-months old.

(*iii*) Rate of growth. The rate of growth on both the media, under identical conditions of light and temperature as stated before, was rather slow to start with but after a week it increased and the daily increment in diameter of Petridish cultures were determined. The radial growth in all cases was unequal and as such average measurements were taken. On malt agar both at 18°C. and 23°C. (Pl. V, fig. 7) the daily increment of growth in diameter varied between 13 and 14 m.m. while on potato-dextrose agar, it varied between 10 and 14 m.m.

(*iv*) Hyphal characters (Text-fig. 1, j–l; Pl. V, fig. 3).

Aerial mycelium. Hyphae hyaline, more or less straight or slightly flexuous, remaining unbranched for a considerable distance (text-fig. 1,j) but often becoming very flexuous and closely branched (text-fig. 1, k), usually with simple clamp-connections at every septum, paired clamp-connections few, often developing into new hyphae, mostly 1.5 to 3μ sometimes 3.5–4.5μ wide, with dense to sparsely granular contents, sometimes empty. *Submerged mycelium.* Hyphae hyaline, sparingly branched, usually flexuous, simple clamp-connections (text-fig. 1, b) frequent but not so numerous as those in aerial hyphae, distantly septate, mostly 3.3.5μ sometimes 1.5–2.5μ wide; a few prominent flexuous usually unbranched hyphae with densely granular, highly refractive contents present, without clamp-connections, about 5–7.5μ occasionally 3.5μ wide; on being mounted in lectophenol its contents become more or less homogeneous and the walls of the hyphae bulge out at places to form lateral swellings (Pl. V, fig. 3).

Production of fruit-bodies

It has already been stated that the two fungi isolated from the infected trees were identified as *S. sanguinolentum* and *P. mitis* respectively, by studying and comparing their cultures with the polysporous cultures of the same already made available for the purpose. Since the identification of unknown cultures largely depends upon their forming characteristic fructifications, various attempts were made to obtain these in pure cultures of the two isolates so that they could be easily recognised. Both the isolates, however, did not form fructifications on various media tried such as potato-dextrose agar, malt agar and oatmeal agar which were subjected to various conditions of moisture, light and temperature within the laboratory. Only *S. sanguinolentum* formed small resupinate fructifications on sterlized wood blocks of Norway spruce within 2 to 3 months after inoculation (Pl. IV, fig. 2). These wood block cultures were kept in darkness and at a constant temperature of 23°C. in an incubator. Similar wood block cultures of *P. mitis* did, however, remain sterile. Eventually the medium for developing sporopheres recommended by Badcock (1941) was tried with considerable success. Dry sawdust of readily decaying wood such as beech and spruce were separately mixed with Badcock's 'accelerator' in various proportions (5% to 40% by weight), the principal ingredients being maize-meal and bone-meal. The mixtures, after being soaked in tap water, were put in Petri dishes, test tubes (20 × 4 c.m. and 15 × 2 c.m.) and Badcock's apparatus (1943) and the various devices described by him for obtaining an atmosphere of relatively high humidity around the developing sporophore, were tried. This was found to be necessary as Badcock rightly pointed out that no single method could be expected to induce fructifications in all species of wood-rotting fungi equally, for they behave very differently under natural conditions. After sterilization the media were inoculated with the isolates and the cultures were kept in darkness at room temperature (18°C. to 21°C.) inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when the mycelia entirely covered the surface of the medium or reached the ends of the tubes, all the cultures were removed and placed in strong diffused light on a table about 8 ft. from a large window of the laboratory. Both the fungi produced exceptionally rapid and luxuriant growth on the sawdust medium, particularly on those containing 30% or 40% by weight of the 'accelerator'.

In all cases, *S. sanguinolentum* began to form either resupinate or typical effuso-reflexed fructifications within 2 to 3 months after inoculation (Pl. IV, fig. 1). When the medium in large tubes was completely permeated with compact mycelium, the cotton plugs were removed and each was supported in an inclined position between two Petri dish lids, the lower being filled with water. A thick pad of absorbent cotton wool was placed within the mouth of the tube and was never allowed to dry out. Within a few days, when vigorous surface growth developed, the pad was gently pushed down so as to touch the medium. The tube was then placed in such a position so that light from the window could fall directly on its open mouth. In this way several effuse-reflexed fructifications were obtained in about 5 to 6 months after inoculation. Although there was

risk of contamination, this method proved to be very successful. In Badcock's apparatus small resupinate fruiting bodies developed near the rim of the test tube within the flask but the growth was very slow. Cultures in Petri dishes in an advanced stage of growth were exposed by removing the lids to humid atmosphere and these also fructified and produced resupinate fruiting bodies within 2 to 3 months after inoculation, particularly near the edge of the lid. It was noted that in case of *S. sanguinolentum* the medium containing 5% 'accelerator' was found to be most satisfactory for the production of sporophores. The higher the percentage of the 'accelerator' the greater was the vegetative growth with consequent delay in producing fruting bodies.

Great difficulty was, however, experienced in obtaining fruit-bodies of *P. mitis* although various devices recommended by Badcock were tried. Under laboratory conditions of light and temperature the mycelium covered the surface of the medium with white and compact growth and crept over the rim of the lower lid of the Petri dish forming somewhat compact, tough, irregular and skin-like mat. At this stage the upper lids were carefully removed and the cultures were kept inside a bell-jar in which a relatively high humidity was maintained by keeping a dish of water exposed within it. Within a fortnight short stalks began to develop near the edge but these did not expand further to form pilei. About the middle of October, 1950, some of these cultures (about 4–5 months old) with rudimentary stalks were then kept in the open outside the laboratory, under a moist bell-jar below a shade, in order to avoid direct sunlight. A wad of moist absorbent cotton wool was placed on the culttre in close contact with the undeveloped stalks and never allowed to dry out. The temperature was daily recorded and it varied between 5°C. and 15°C. till the middle of November. Within a fortnight numerous stalks began to develop on the outer edge of the skin-like mat and within a month their tips expanded and formed pilei with rudimentary gills on their under surface (Pl. II, fig. 6). In this way several crops of fructifications of *P. mitis* were obtained. It was found that temperature of the laboratory (18°C.—21°C.) and incubators had a retarding influence on the production of sporophores. Given all other necessary conditions, a range of temperature varying between 8°C. and 15°C. was found suitable for their development. When the temperature fell below 8°C. development of fructifications was gradually stopped. *P. mitis* appeared to be very sensitive to temperature conditions and it was also necessary to increase the amount of 'accelerator' to 40% by weight for ready development of the sporophores. Fructifications of both the fungi when sectioned showed well-developed hymenia with normal basidia and basidiospores.

Fructifications of both *S. sanguinolentum* and *P. mitis* began to develop readily when small sections of freshly felled infected trees were brought into the laboratory and kept outside in the open, during the months of November to January (Pl. I, fig. 4). Occasional showers kept the poles moist and a comparatively low temperature was found to be suitable for their development. In this respect *P. mitis* appeared to be very sensitive to outside temperature for it was also observed that a range of temperature between 8°C. and 15°C. was found favourable for their development.

During the rest of the months the fungi lived well in the moist wood but did not fructify at all. Similar infected poles were also kept moist in diffused light and temperature (18°C.—21°C.) of the laboratory. *P. mitis* formed dense strands of white mycelium on the surface of the wood (Pl. II, fig. 4), but did not produce any fruit-body. Small resupinate fructifications of *S. sanguinolentum*, however, developed at the cross-section-ends of the poles but their growth appeared to be very slow.

Decay of Spruce

Microscopic details of the rot

In order to find out the extent and effects of the mycelium within the host, pieces of wood in various stages of decay were sectioned both free hand and with the microtome. Transverse, radial and tangential sections of the wood, about 15–20μ thick, were cut without any special treatment, from freshly felled poles. For ordinary purposes free hand sections were found to answer the requirements well to ascertain the presence of mycelium within the host tissue. Differential stains such as those recommended by Hubert (1922), Cartwright (1929) and others were tried but the method proposed by Cartwright was found to be very satisfactory in rendering the hyphae visible and easy to manipulate.

In an early stage of decay caused by *S. sanguinolentum*, the mycelium was often scanty but later it was distributed throughout all the elements (Pl. V, fig. 6) being particularly plentiful in the medullary rays which seemed to be the first element of the wood to be attacked. The hyphae, sometimes filling entire cavities of the elements were of two kinds,—firstly, rather fine, profusely branched, hyaline, about 1–2μ across and secondly, a coarser hyphae (Pl. VI, figs. 1–3) of about 3–5 (6)μ wide. The larger hyphae were hyaline or pale yellow in colour, occasionally with reddish-brown contents and usually bearing large, simple or double clamp-connections (Pl. VI, figs. 2 & 3). Similar double clamp-connections on coarser hyphae were recorded in two samples of spruce by Jorstad and Juul (1931). Clamp-connections were rare on the finer hyphae. The finer hyphae appeared to be more numerous and probably were the first to invade the uninfected parts. These could be definitely demonstrated in properly stained preparations even in an incipient stage of decay in which the coarser hyphae with double clamp-connections had not been observed. The hyphae at first ramified through the pits but later directly through the cell-walls forming numerous bore-holes (Pl. VII, fig. 4). The much branched finer hyphae in passing through the cell-walls exhibited little or no diminution in diameter while the larger hyphae when passing through the cell-walls, became markedly attenuated or constricted towards the apex. The photograph (Pl. VI, fig. 4) submitted in evidence shows clearly the bore-holes slightly wider in diameter than the contained hyphae; these bore-holes were formed in advance by enzyme action at the point of contact of the hyphae. The bore-holes were usually circular or somewhat cylindrical and plentiful in an advanced stage of decay. Many of the ray parenchyma cells and resin canals (Pl. V, fig. 5) were filled with

closely interwoven mycelium associated with a yellowish gum-like substance. These were also occasionally present in the tracheids. Similar plugs of gum-like materials giving the appearance of a zone-line had also been observed by Cartwright and Findlay (1946). In passing through the bordered pits the hyphae destroyed the closing membranes, thus enlarging the opening. In an advanced stage of decay the walls of the bordered pits were dissolved by enzyme action as shown by the smooth contours of their surfaces and their circular form. The wood in an advanced stage of decay (Pl. VI, fig. 6) showed that the secondary walls of the tracheids had been gradually dissolved outwards towards the middle lamella and this appeared to be a slow process.

The microscopic details of the rot caused by *P. mitis* were more or less similar to those caused by *S. sanguinolentum* except for the following differences. The hyphae were exceedingly narrow, much branched, hyaline, about 1–1.5μ across and plentiful in the decayed wood (Pl. VII, figs. 1 & 2) but slightly wider hyphae up to 3μ wide were also present. Simple clamp-connections (Pl. VII, fig. 2) were very numerous, almost at every septum: the modes of hyphal penetration were through the pits as well as through the cell-walls (Pl. VII, fig. 2). The bore-holes (Pl. VII, fig. 2), exceedingly small, more or less circular in outline, were very numerous. The walls of the bordered pits (Pl. VII, fig. 3) were broken down early. Entangled masses of hyphae were quite common in the tracheids. The ray parenchyma cells were filled with interwoven hyphae and yellowish or brownish gummy materials but the latter were rare in the tracheids.

Microchemical studies

Microchemical tests for lignin and cellulose in the sound and partly decayed wood have been recorded. This was done in the usual way by staining the sections of wood with phloroglucin and hydrochloric acid for lignin and with chlor-zinc-iodine for cellulose. The combination stain, safranin and fast green which differentiates lignified (red staining) and unlignified (green staining) structures and has superseded the old safranin and haematoxylin combination, was tried. Various other microchemical stains were also employed, most of which agreed closely with the changes indicated by the use of above-mentioned stains. It is ,however, not wished to draw any definite conclusion from these stain-reactions alone, since it has been recently shown (Harlow 1928; Hirt 1927) that these microchemical stains are not infalliable indicators of the chemical changes that take place during the process of decay in the wood. Nevertheless, these stains are still used in such work to indicate the presence of lignin and cellulose.

In the normal wood the middle lamellae of all the elements stained red. The secondary walls of the wood tracheids were partly light red and partly green but sometimes entirely green. The secondary walls of the ray cells were however, occasionally partly green. The secondary walls of the epithelial cells of the resin canals were so thin that possibly

the red tinge came from heavily stained middle lamellae. The bordered pits were mostly pinkish but with a faintly greenish tinge near the aperture.

In the decayed wood a light red to pink colour was more noticeable over the entire sections. The middle lamellae remained red but they were light red to somewhat pinkish in the way and epithelial cells of the resin canals. The secondary walls of all the elements were predominently pinkish but occasionally light red, and faintly greenish to almost colourless surrounding the lumen. The bordered pits were often entirely greenish in colour.

Table 1. *Results of staining with safranin and fast green for cellulose and lignin in normal and partially decayed wood of* Norway spruce (*Picea excelsa*)

		CELLULOSE (Fast green)	
Elements	Normal Wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Secondary walls surrounding the lumen either green or partly green and extending up to middle-lamella, occasionally almost entirely green; bordered pits often faintly green surrounding the aperture.	Secondary walls round the lumen faintly green, often partly light green, and extending up to middle lamella.	Secondary walls in the summer wood, occasionally partly pale green; spring wood mostly pale green round the lumen; bordered pits often greenish.
Ray cells	Secondary walls often partly greenish.	Occasionally faintly green or colourless.	Occasionally colourless.
Resin ducts	No reaction	No reaction	No reaction
		LIGNIN (Safranin)	
Elements	Normal Wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Primary walls deep red; secondary walls lighter red; bordered pits mostly pink.	Primary walls red; secondary walls mostly pink towards the middle lamella.	Primary walls red; secondary walls almost wholly light red to pink, occasionally partly so; bordered pits often pinkish.
Ray cells	Primary walls red; secondary walls light red.	Primary and secondary walls mostly pink.	Primary walls red or light red; secondary walls mostly light red to pink.
Resin ducts	Red	Pink	Light red.

Certain investigators in using safranin and fast green as a combination stain state that the red colour indicates the presence of lignin and that the unlignified tissues are stained green. Accordingly, it can be broadly

interpreted that the fungi in question gradually delignify all the elements but also utilize to a great extent the cellulosic materials of the wood. It would be hardly wise to draw any definite conclusion from the above staining reactions, since when other stain combinations such as gentian violet and Bismarck brown are used the partly brownish colour of the epithelial cells do not necessarily indicate lignin.

Table 2. *Results of staining for cellulose and lignin in normal and partly decayed wood of* Norway spruce (*Picea excelsa*) *with chlor-zinc-iodine and phloroghicin-HCl respectively*

		CELLULOSE (Chlor-zinc-iodine)	
Elements	Normal wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Secondary walls near the lumen mostly blue, sometimes light blue, in summer wood less distinct; primary walls occasionally faint blue; bordered pits partly faint blue in most cases.	Secondary walls near the lumen light blue mostly, particularly in the spring wood; primary walls occasionally partly light blue; this reaction more conspicuous than that in normal wood; bordered pits faint blue at places.	Secondary walls partly faint blue at places; reaction more feeble than that in normal wood; primary walls at places faint blue in spring wood.
Ray cells	Secondary walls partly faint blue, particularly of the ray tracheids.	Secondary walls partly light blue.	Secondary walls mostly colourless.
Resin ducts	Partly faint blue	No reaction	No reaction
		LIGNIN (Phloroghicin-HCl)	
Elements	Normal wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Primary walls deep red; secondary walls lighter red near the middle lamella to pink near the lumen; often entirely light red or pink; bordered pits pink.	Primary walls red or pink; secondary walls mostly pink; light pink particularly near the rays; bordered pits pink or colourless.	Primary walls red or pink; secondary walls light red or pink, occasionally faint pink; bordered pits often colourless near the aperture but mostly pink.
Ray cells	Primary walls red; secondary walls pink.	Primary walls pink; secondary walls pink; occasionally both colourless.	Primary walls pink; secondary walls light pink; occasionally both faintly pink.
Resin ducts	Mostly red	Pink, occasionally red	Mostly red

Phloroglucin–HCl.—In normal wood the middle lamellae of all the elements stained a deep red. The secondary walls changed from light red near the lamellae to somewhat pink near the lumen. Occasionally, they were entirely light red or pink. The secondary walls of the ray cells

and the walls of the bordered pits were pink. The epithelial cells of the resin canals remained red.

In the decayed wood the middle lamellae of the wood tracheids remained red or became pink and those of the ray cells mostly pink. The secondary walls of the wood tracheids and ray cells mostly became entirely pink or lighter in colour but occasionally remained light red. The middle lamellae and secondary walls of some of the ray cells became colourless. The walls of some of the epithelial cells turned pink.

Chlor-zinc-iodine.—In normal wood the middle lamellae seldom showed faint blue colouration. The secondary walls of the wood tracheids were mostly blue or light blue and those of the ray cells, faint blue. This was particularly the case with the ray tracheids. In the summer wood this reaction was less distinct. The walls of the epithelial cells were partly faint blue.

In the decayed wood occasionally the middle lamellae remained partly faint blue. In wood decayed by *S. sanguinolentum* the secondary walls of the wood tracheids showed intense cellulose reaction. They were mostly light blue, particularly in the spring wood and partly light blue in ray cells. The bordered pits were faint blue, particularly in the spring wood. In wood decayed by *P. mitis*, on the other hand, the secondary walls of the wood tracheids showed feeble cellulose reaction, being partly faint blue at places. The secondary walls of the ray cells were mostly colourless. The bordered pits remained colourless in both cases.

The pink colouration of the secondary walls of the wood tracheids and ray cells when treated with phloroglucin-HCl and the blue or light blue colouration in response to chlor-zinc-iodine, indicate that they are only moderately lignified. The chemical changes indicated by these staining reactions clearly show that there was delignification of the lignified parts of the secondary walls associated with the digestion of cellulosic materials of the wood. In wood decayed by *S. sanguinolentum* some of the secondary walls showed only pale pink colouration but mostly stained light blue throughout, suggesting partial removal of lignin from those walls. This reaction was more conspicuous than that in the normal wood. In case of *P. mitis* there was least cellulose reaction and the walls turned red or pink, suggesting the presence of more lignin in these walls.

Sulphuric acid test.—Sections of normal and decayed wood corresponding to those used for microchemical stains were placed upon slides and treated with 72% sulphuric acid as suggested by Ritter (1925). Immediately, in sections of normal wood, the cells swelled up rapidly and broke apart into fragments in the spring wood. The secondary walls of the wood tracheids became transluscent and partly dissolved while the network of the middle lamellae remained intact in the summer wood but torn apart in the spring wood. The ray elements remained completely unaffected but their secondary walls became swollen and partly dissolved. The violent action during the process was due to the presence of considerable amount of cellulose in the cell-walls of the sound wood.

The swelling of cellulose preceeding dissolution forced all the elements apart in the spring wood.

In sections of wood decayed by both *S. sanguinolentum* and *P. mitis* fragments of secondary walls of the wood tracheids were dissolved leaving the network of middle lamellae and wood elements in place on the slide. In both cases the secondary walls of the tracheids became swollen, partly dissolved and semi-transparent. The network of middle lamellae mostly remained in tact though broken off at places in the spring wood. There was much less distortion of the elements in spring wood than those in the normal wood. The ray elements mostly remained unaffected and portions of their secondary walls were the only parts dissolved.

It may be concluded that the less violent action and consequently less distortion of the wood elements in the decayed wood were due to the presence of less cellulose than that in the sound wood. This is particularly true in case of wood decayed by *P. mitis*. Though not conclusive in itself, this test at least indicates the presence of less cellulose in the wood decayed by *S. sanguinolentum* and *P. mitis* respectively than that in the normal wood.

Decay resistance tests

In order to determine the natural resistance and progress of decay of spruce samples caused by *S. sanguinolentum* and *P. mitis* separately, under controlled condition in the laboratory, wood-block cultures of these fungi were made in the following way. In general, the method recommended by Baxter (1925) was employed with slight modification.

Small bits of actively growing mycelia were transferred to sterile 2 per cent malt agar slants in 1000 c.c. Erlenmeyer flasks. Each flask contained 250 c.c. of the medium which was slanted after sterilization. After inoculation the flasks were incubated at 23°C. in darkness. Within a week the mycelia covered the surface of the slants and were ready to receive the wood blocks.

The wood blocks measuring $2'' \times \frac{1}{2}'' \times \frac{1}{2}''$ were obtained from sound wood of Norway spruce. These were planed and serially numbered on all faces and dried to a constant weight in an oven maintaining a temperature of 60°C. A comparatively low temperature was preferred to avoid the harmful effects of rather high temperatures on the composition of the wood blocks. Before sterilization these test-pieces were thoroughly soaked in distilled water, three such pieces were put in each Roux tube having distilled water at the bottom, plugged and sterilized in an autoclave at 15 lbs. pressure for 10 minutes. The idea of sterilizing the wood blocks in this way was to keep the atmosphere around them more or less saturated during the process so that little loss of water could take place from the wood blocks and consequently lesser time was necessary for sterilization as pointed out by Chidester (1937, 1939). The test-pieces were then taken out individually from the Roux tubes by a sterile forceps and exposed to fungal action by placing them on cultures of *S. sanguinolentum* and *P.*

mitis (Pl. VIII, figs. 1,2). These flasks, each containing six samples of wood were kept at a constant temperature of 23°C. in darkness.

In order to obtain a significant loss in weight the periods of exposure to fungal attack were 4 months and 8 months. After completion of the tests the flasks were opened, the superficial mycelium was carefully removed without damaging the wood-blocks (Pl. VIII, figs. 3 & 4) weighed and again dried to a constant weight in an oven at 60°C. The final dry weight subtracted from the original dry weight gave the loss in weight of the wood blocks due to decay in 4 and 8 months. The results are presented in Table 3.

The average moisture-content of the wood blocks before they were exposed to fungal action was determined by weighing another set of 12 blocks, soaked and sterilized in the same way. The final dry weights when subtracted from these gave the initial moisture-content of the wood-blocks before the experiment. This was necessary to keep the moisture-content of the wood-blocks much above the 'fibre-saturation-ponint' which was necessary for the activity of the wood-rotting fungi. In this way the moisture-content of the inoculated wood-blocks after the tests was also determined. The initial moisture-content of the wood blocks before the experiment averaged 158%. After both 4 and 8 months' test it was found to lie between 52–62%.

Table 3. *t-test of comparison of loss in dry weight of wood blocks of* Norway spruce *exposed to* S. sanguinolentum *and* P. mitis *after* 4 *and* 8 *months.*

Test Fungus	Months	Number of Replicates	Mean loss in dry wt. (%)	S.E.	Comparison between Means	*t*	Probability
S. sanguinolentum	4	12	14.6 A	±1.25	A and B	14.23	<.01
	8	12	29.0 B	±3.11	C and D	51.89	<.01
P. mitis	4	12	12.5 C	±1.21	A and C	3.12	<.01
	8	12	23.8 D	±2.16	B and D	5.40	<.01

It is evident from the *t* values of Table 3 that the loss in weight due to *P. mitis* is significantly less then that caused by *S. sanguinolentum*. The loss in weight after 8 months is double of that after 4 months in each case, and is highly significant. It can, therefore, be concluded that the wood has low resistance to decay. Accordingly, spruce wood falls under Findlay's (1938) 'non-resistant group as this class undergoes average losses from 10–30% during four months' test.

Field inoculations

Inoculation experiments were carried out with young twigs in the laboratory and also with trees in the field. In the former case the method described by Brooks and Moore (1923) was followed. In this experiment, 2 to 3-years-old twigs of Norway spruce, about 4″ along, were kept with their ends in water in the laboratory, their upper ends were cut off and spore-suspensions of *S. sanguinolentum* and *P. mitis* in sterile water were applied separately to the freshly exposed surface and never allowed to dry out. These were placed under a bell-jar in which a relatively high humidity was maintained. Longitudinal sections through the inoculated ends of the twigs were made at intervals of 24, 48, 72 and 96 hours after the spores had been added. Sections cut after a period of 24 to 48 hours showed that the majority of the spores had germinated on the surface and had sent hyphae down into the elements of the wood. A few spores were, however, found to enter through the cut ends of the tracheids and had germinated there. Sections cut at intervals of 72 a d 96 hours indicated that the spores had formed vigorous mycelia and invaded all the elements of the twig. Since these twigs could not be kept in an actively growing condition, their invasion by *S. sanguinolentum* and *P. mitis* could not be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores some how alight on the surface of the wound, they can germinate under suitable conditions producing germ-tubes which in some way pass down into the elements of the wood.

Field inoculations on living trees were started in early March, 1950, at Glentress forest. Two inoculations were made on 50 to 60-years-old vigorously growing Norway spruce with each fungus and with equal number of controls below. The operation involved was to make a cross-incision through the bark on the trunk and the trees were inoculated by transferring actively growing mycelium into the wound by carefully lifting a flap of the bark still attached to it.

Prior to inoculation the surface of the bark was wiped out with absolute alochol and subsequently washed with sterile distilled water in order to free the surface from contamination as far as practicable. The flap was then covered with moist, absorbent, sterile cotton wool with a piece of thick paper over it in order to prevent drying out and firmly bound with a string. Towards the end of March the trees were examined. After removing the string and the cotton wool it was found that in both cases the trees seemed to have taken the infection. The inoculated areas were distinctly bulged out with conspicuous secretions of resin. The controls, on the other hand, remained flat, rather depressed and there was little resin flow. These trees were again inspected in July and it was observed that in both cases the controls remained flat and were healing up satisfactorily. There was very slight resin secretion within the cut. In case of *S. sanguinolentum* the wounds were gaping open, looked more or less swollen, somewhat spindle-shaped and there was copious secretion of resin running down the stem (Pl. VIII, fig. 6). From the appearance of the bark it looked as if the infection had spread on the left side about

$1\frac{1}{2}''$ from the point of infection. Infections with *P. mitis* also looked somewhat oval and the resin was running down the stem (Pl. VIII, fig. 5). The infection appeared to have spread about an inch from the point of infection. In November, after 8 months, the trees were examined and the progress of infection was noted. All controls healed up. In case of *S. sanguinolentum* the bark was gradually sinking and there was more resin flow from the infected wounds than those caused by *P. mitis*. From the outward indications it could be said that both the fungi had infected the living trees through wounds, although *S. sanguinolentum* seemed to be more active of the two pathogens and produced more resin which ran down the stem. At this time no fruiting-bodies of the fungi were observed and this was only to be expected since both the fungi was slow growers within the tissues of the host.

In March, 1951, the trees were examined and one of them felled. The internal conditions of the inoculated areas were microscopically examined: mycelia of both *Stereum sanguinolentum* and *Pleurotus mitis* respectively were present in the elements of the wood. Copious gum formation had taken place within the medullary rays.

Microscopic examination of the infection by *Stereum sanguinolentum* showed characteristic reddish-brown rot in the sapwood. The mycelium had spread approximately eight inches both above and below the point of infection. Spread in the transverse direction was about half an inch. In the infection with *Pleurotus mitis* the yellowish brown rot is confined to the sapwood and extended about two inches both above and below the point of inoculation; the spread in the radical direction is about one quarter of an inch. Subsequeutly re-isolations of the two pathogens from infected tissues were made and these agreed with the original cultures of *S. sanguinolentum* and *P. mitis* in all essential details.

Discussion

This study has been undertaken to obtain information of a serious disease causing bark necrosis on the trunk on Norway spruce at Glentress forest, near Peebles, in Scotland. The injuries are restricted to the stem mostly within the pruning limits and have been diagnosed as having arisen from the attack of *Stereum sanguinolentum* and *Pleurotus mitis*. These fungi have entered through the dead branches or other injuries such as pruning cuts that might have taken place at the beginning or the end of the growing season. Although similar injuries on Norway spruce have been attributed by Day (1950) as entirely due to frost damage, there is no such evidence on trees under consideration. Gäumann (1950) has described similar injuries in Switzerland on the bark of Norway spruce and silver fir, in a situation exposed to frequent hailstorms and strong electrical discharges which killed strips of cambium: infection with *P. mitis* followed. Careful examination of the infected trees and the anatomy of the injuries did neither reveal the presence of frost cracks and frost rings in their wood nor was there any sign of bark injury due to electrical discharges. In addition, during the past few years there is no evidence

that frost injury has really occurred in the crop as a whole. On the other hand, close examination of the wounds reveals that the cambium has been killed near the pruned branches and the indications are that the causal agents after entering through the wound in the dead branches or pruning cuts, have killed the cambium and spread from the periphery to the centre of the wood, thereby rendering the wood unfit for commercial purposes. In the absence of any other evidence, it may be taken that these wounds have served as initial portals of entry. Both the fungi are common in the plantation as saprophytes on fallen branches lying on the floor of the forest during the colder months of autumn and the early part of winter.

Low air-temperatures appear to be favourable for the development of the fruiting bodies and during the spells of mild weather, i.e., late autumn and early spring when the trees are in a dormant condition, the spores after being carried away by the wind alight on the cut surfaces of the pruned branches where they germinate and penetrate the wood. It has been experimentally proved that a comparatively low temperature is necessary for the development of the fruit-bodies, particularly in case of *P. mitis*, and that the spores can germinate within a short time on the cut surfaces and infect the twigs under moist conditions. Inoculation experiments on living trees carried out in the field during early spring have shown that both the pathogens can infect standing trees through wounds in the bark and from the results of inoculation they can be regarded as wound parasites on normal vigorous trees. Although no infection experiments have been carried out with dead branches, it can be assumed that presumably they can also infect them as they can live well in the wood of the fallen branches. As long as the trees are in sap and the air-temperatures are high, the fructifications cannot develop and consequently there is comparatively less chance of any infection taking place during the period. In summer, the fungi thrive well in the dead branches in a moist cool situation. Experiments with infected trunks point to the conclusion that the fungi can live within the wood throughout the year and begin to fructify only during the months of October and November, when the outside temperature is low and favourable for their development.

Both *S. sanguinolentum* and *P. mitis* have been isolated from standing trees and found to be the cause of the disease. Previous investigators of spruce have failed to record that *P. mitis* can cause an important decay in wood of living trees in Great Britain. In transverse sections of the infected poles, the general appearance of the rots in the early stages of decay caused by *S. sanguinolentum* and *P. mitis*, is very similar and in this study both decays were at first thought to be caused by the former fungus until cultural and microscopic investigations proved differently. This has emphasized the importance of conducting cultural studies in support of decay determinations which are often based on gross appearance of the rots. It has, however, not been possible to find out whether both the pathogens have infected the trees at about the same time or whether one of them has entered first and weakened the tree, thus preparing the ground for a secondary infection with the other fungus. At the present moment it is difficult to estimate the relative importance of the two fungi

in bringing about infection and decay in living trees and the nature of interactions existing between them but this association of two pathogens has no doubt greatly enhanced the importance and complex nature of the problem. However, both the fungi have been isolated not only from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. From the number of isolations so far made, it is apparent that *S. sanguinolentum* is more widely distributed within the host tissues than *P. mitis*. Further investigations in future with large number of infected trees would possibly throw some light as to their distribution within the host plant. In consequence, the amount of damage in the field caused by the pathogens could not be determined. But experiments on durability tests on spruce timber carried out in the laboratory have shown that spruce timber is 'non-resistant' to decay caused by *S. sanguinolentum* and *P. mitis*. There is no doubt, however, that such injuries caused by these fungi, will cause serious deterioration in the value of timber and the damages caused by them may become very serious.

Control measures

As wounds on the stem appear to be closely associated with the disease, the treatment should be preventive and not curative. Although further infection experiments on living spruce by these fungi, following pruning of living branches, are necessary, control measures based on general principles can be suggested and these may be put into effect in the affected areas in order to prevent spreading of the disease. Great care should be taken during pruning operation and thinning in order to avoid injuries caused by careless work. Pruning should be done close to the stem but not so close as to damage the bark and no projecting stubs should be left. The pruned surface should be smooth and covered with paint in order to protect the surface from moisture and to prevent penetration of germ-tubes from germinating spores. An alternative method is to apply a fungicide to the cut surface to kill any superficial spore. Removal of all fructifications from the dead branches, as often suggested for preventing dissemination of spores, seem to be an impractiacable proposition, since, tiny fructifications like those of *S. sanguinolentum* would invariably be overlooked during the search. Up to the present the effect on the trees of pruning has been limited almost entirely to the extent of healing process. The evidence which has now been brought forward concerning the infection by fungi as the result of wounding must now be seriously considered.

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EXPLANATION OF PLATES I—VIII

Plate I

Fig. 1. A portion of the plantation at Glentress forest showing infected Norway spruce trees characterised by ill-defined, flat and longitudinal depressions of the bark extending from the butt upwards.

Fig. 2. A longitudinal crack in the depressed bark accompanied by an outflow of resin.

Fig. 3. Fructifications of *Stereum sanguinolentum* on the cross-section-end of a freshly felled trunk of Norway spruce lying on the floor of the forest.

Fig. 4. Effuso-reflexed fructifications of *S. sanguinolentum* have developed on the rotted areas at the cross-section-end of a Norway spruce pole.

PLATE II

Fig. 1. Fructifications of *Pleurotus mitis* on dead branch of Scots pine.

Figs. 2. & 3. Typical fructifications of *P. mitis* on dead twigs of spruce showing various stages of development.

Fig. 4. Mycelial strands of *P. mitis* developed on the surface of the infected pole under moist conditions.

Fig. 5. Fructifications of *P. mitis* with abnormally elongated stipes and mycelial strands formed within the cracks of spruce poles.

Fig. 6. Fructifications of *P. mitis* in culture. (×4).

PLATE III

Figs. 1–4. Cross sections of Norway spruce poles showing various stages of decay; characteristic light to dark irregular or wedge-slaped areas can be seen from the periphery to the centre of the mature wood.

Fig. 5. Longitudinal section of the wood showing the rotted areas.

Fig. 6. Fructifications of both *S. sanguinolentum* and *P. mitis* have developed on respective rots at the cross-section-end of the infested pole under experimental conditions.

Fig. 7. A longitudinal crack in the bark with copious outflow of resin.

PLATE IV

Fig. 1. Effuso-reflexed fructifications of *S. sanguinolentum* formed within test-tubes in artificial culture.

Fig. 2. A resupinate fructification of *S. sanguinolentum* formed on wood-blocks of Norway spruce in culture.

Fig. 3. Culture of *S. sanguinolentum*, 21-days-old, on *potato-dextrose agar* and *malt agar* in darkness at 18°C. (a, c) and 23°C. (b, d) respectively.

Fig. 4. Cultures of *P. mitis*, 21-days-old, on *potato-dextrose agar* and *malt agar* in darkness at 18°C. (a, c) and 23°C. (b, d) respectively.

Fig. 5. Culture of *S. sanguinolentum*, 21-days-old, on *malt agar* at 23°C. in darkness.

Fig. 6. Culture of *S. sanguinolentum*, 21-days-old, on *potato-dextrose agar* at 23°C. in darkness.

Fig. 7. Culture of *P. mitis*, 21-days-old, on *potato-dextrose agar* at 23°C. in darkness.

PLATE V

Fig. 1. Helicoid hyphae of the aerial mycelium of *S. sanguinolentum*. (×400).

Figs. 2 & 4. Aerial hyphae with double clamp connections of *S. sanguinolentum* in culture. (×900).

Fig. 3. Specialized, usually unbranched, flexuous hyphae with densely granular contents; characteristic of the submerged mycelium of *P. mitis*.

Fig. 5. Part of the radial section of the infected wood showing masses of hyphae of *S. sanguinolentum* within the horizontal resin canal. (×100).

Fig. 6. Distribution of the hyphae of *S. sanguinolentum* within the sections of decayed wood; the hyphae are abundant within the tracheids and they have passed mainly through the bordered pits. (×200).

PLATE VI

Figs. 1–3. Hyphae with simple and double clamp connections of *S. sanguinolentum* within the tracheids. (×450).

Fig. 4. Penetration of the cell-wall by the hyphae of *S. sanguinolentum;* the bore-hole is visibly larger than the penetrating hyphae. (×900).

Fig. 5. Part of a *t.s.* showing decay of Norway spruce caused by *P. mitis;* the spring-wood is the most affected part of the section. (×100).

Fig. 6. Part of a *t.s.* showing decay of Norway spruce caused by *S. sanguinolentum;* the elements of the spring-wood are badly damaged.

Plate VII

Fig. 1. Distribution of the hyphae of *P. mitis* within the sections of decayed wood; the hyphae have ramified through the bordered pits and also through the walls forming bore-holes. (×450).

Fig. 2. Hyphae of *P. mitis* within the tracheid showing simple clamp connections; the bore-holes can be clearly seen. (×900).

Fig. 3. Eroded bordered pits of the tracheids due to the activity of *P. mitis.* (×900).

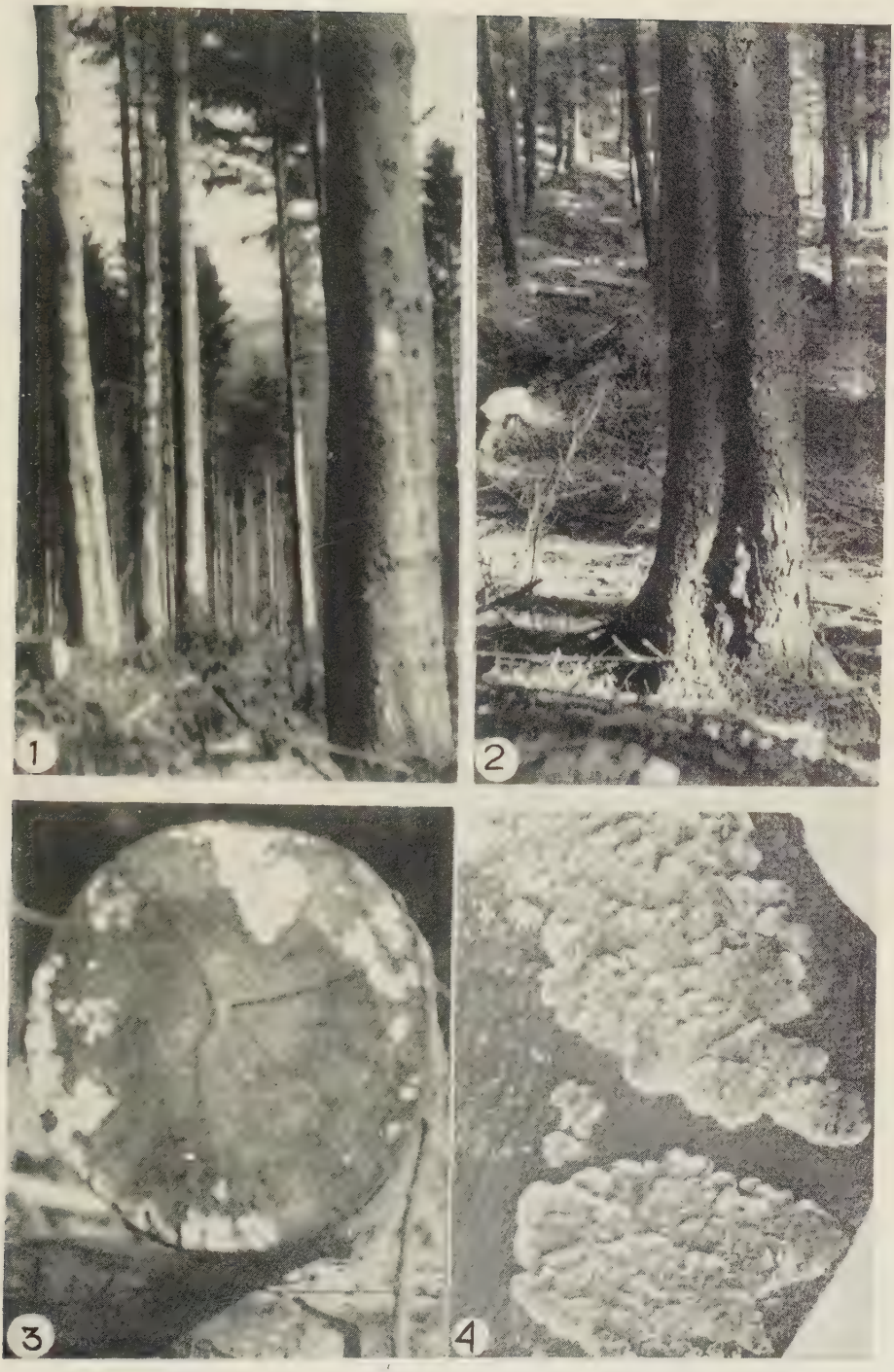
Fig. 4. Hyphae of *S. sanguinolentum* passing through the walls of the bordered 'pits.

Plate VIII

Figs. 1 & 2. Wood-block cultures of *S. sanguinolentum* and *P. mitis* for testing decay resistance in the laboratory; photographed after 45 and 60 days respectively.

Figs. 3 & 4. Wood-blocks of Norway spruce showing decay after 4 months' exposure to *S. sanguinolentum* and *P. mitis* respectively.

Figs. 5–6. Infections on trunk of Norway spruce with *P. mitis* and *S. sanguinolentum* respectively, 5 months after inoculation.



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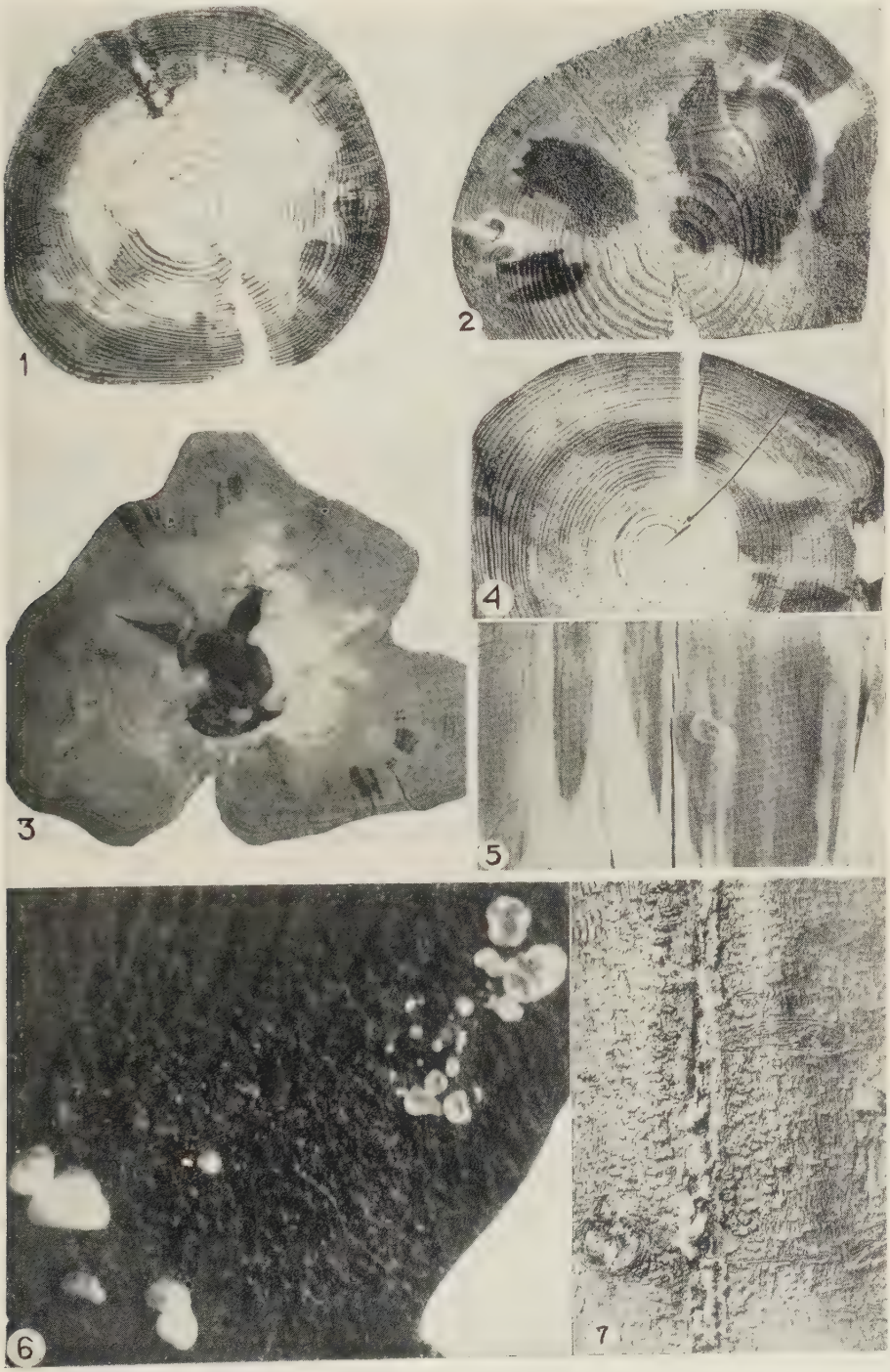
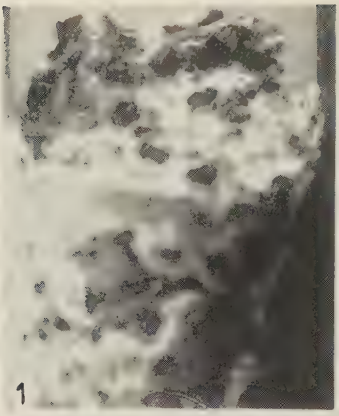
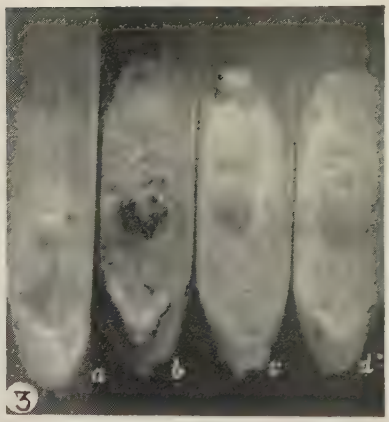
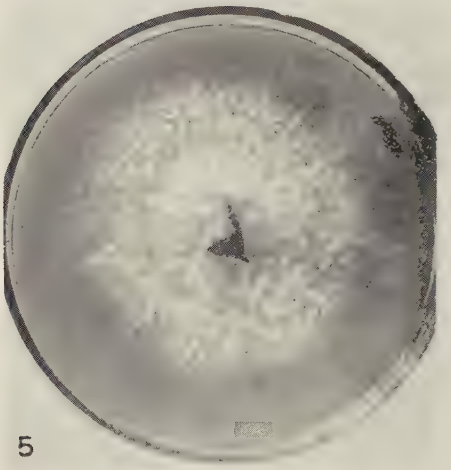


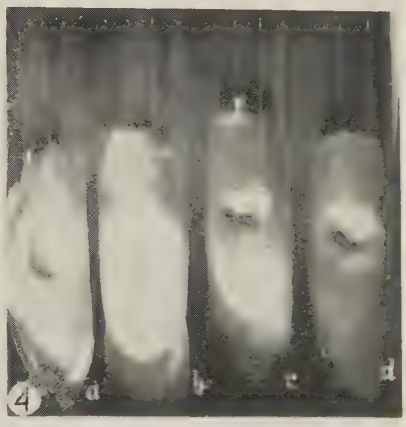
A disease of Norway spruce

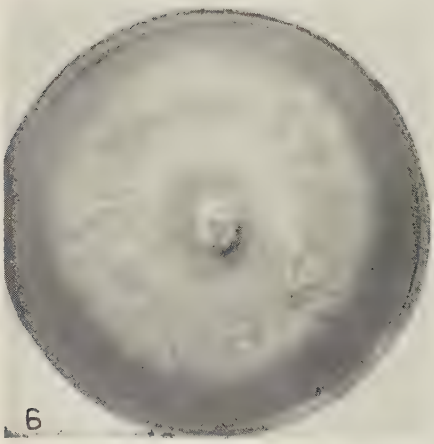
S. N. Banerjee

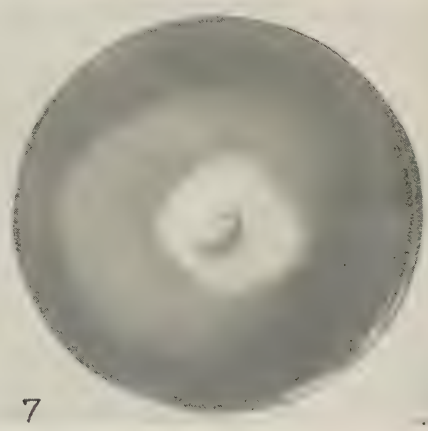












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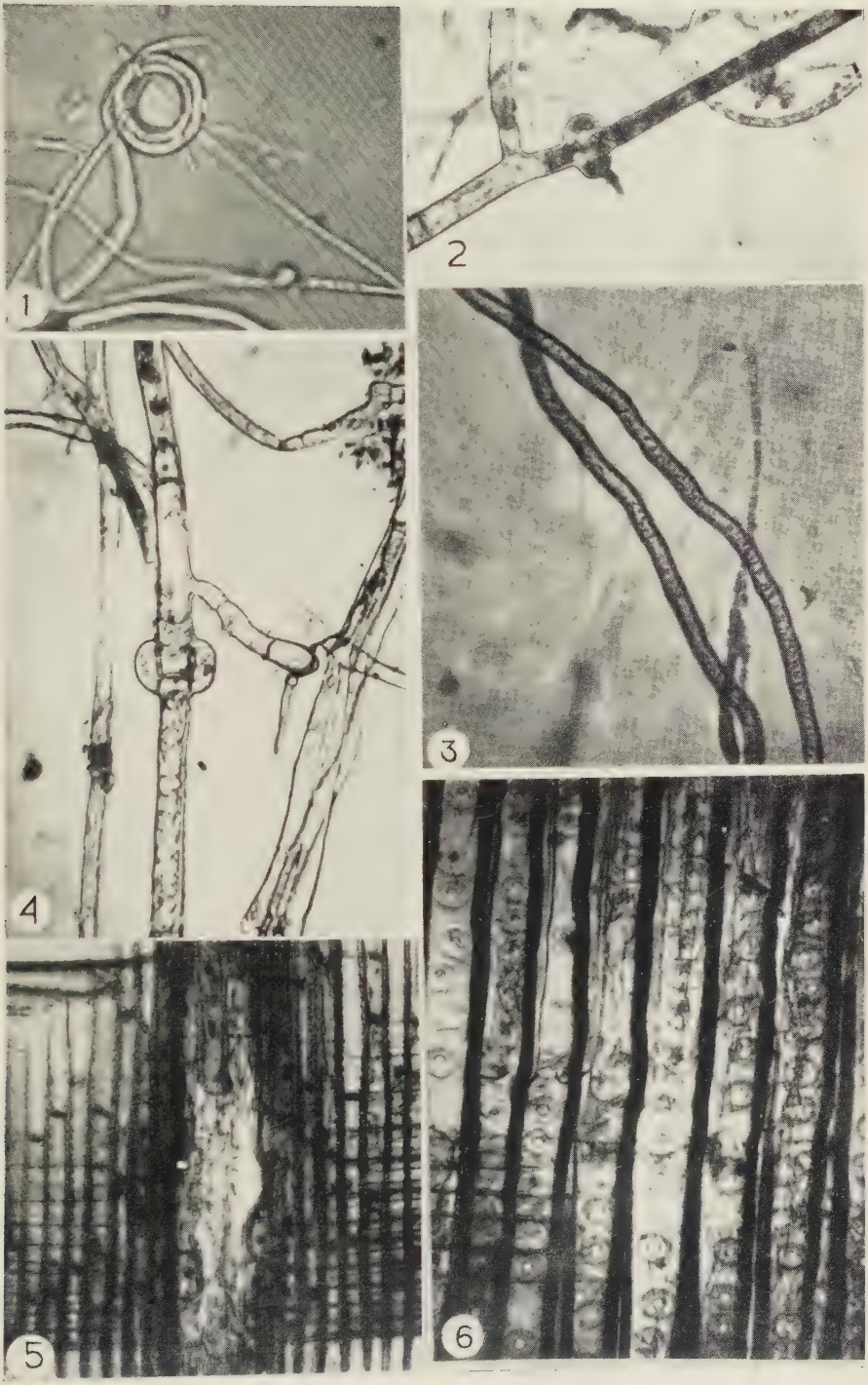

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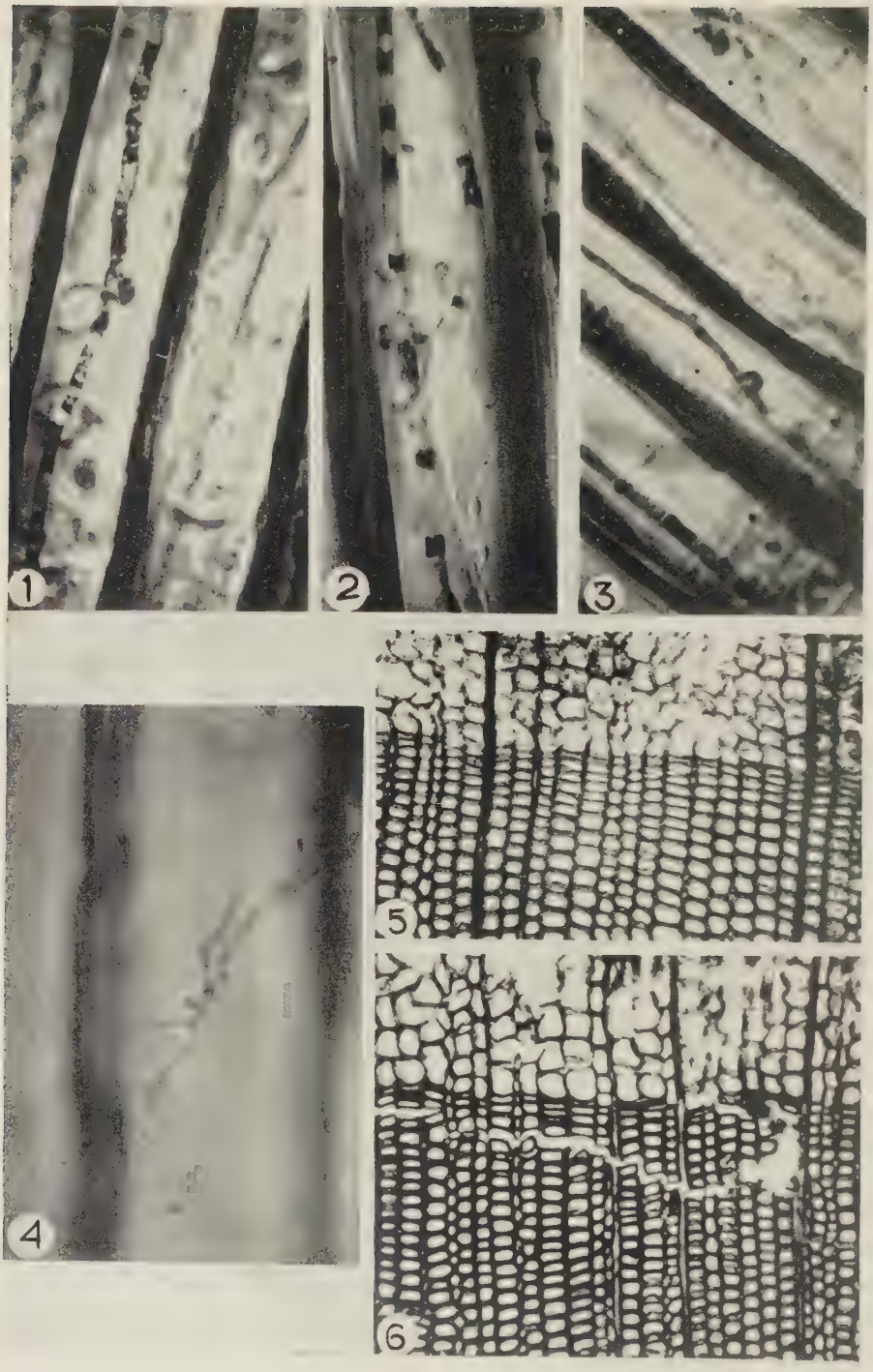

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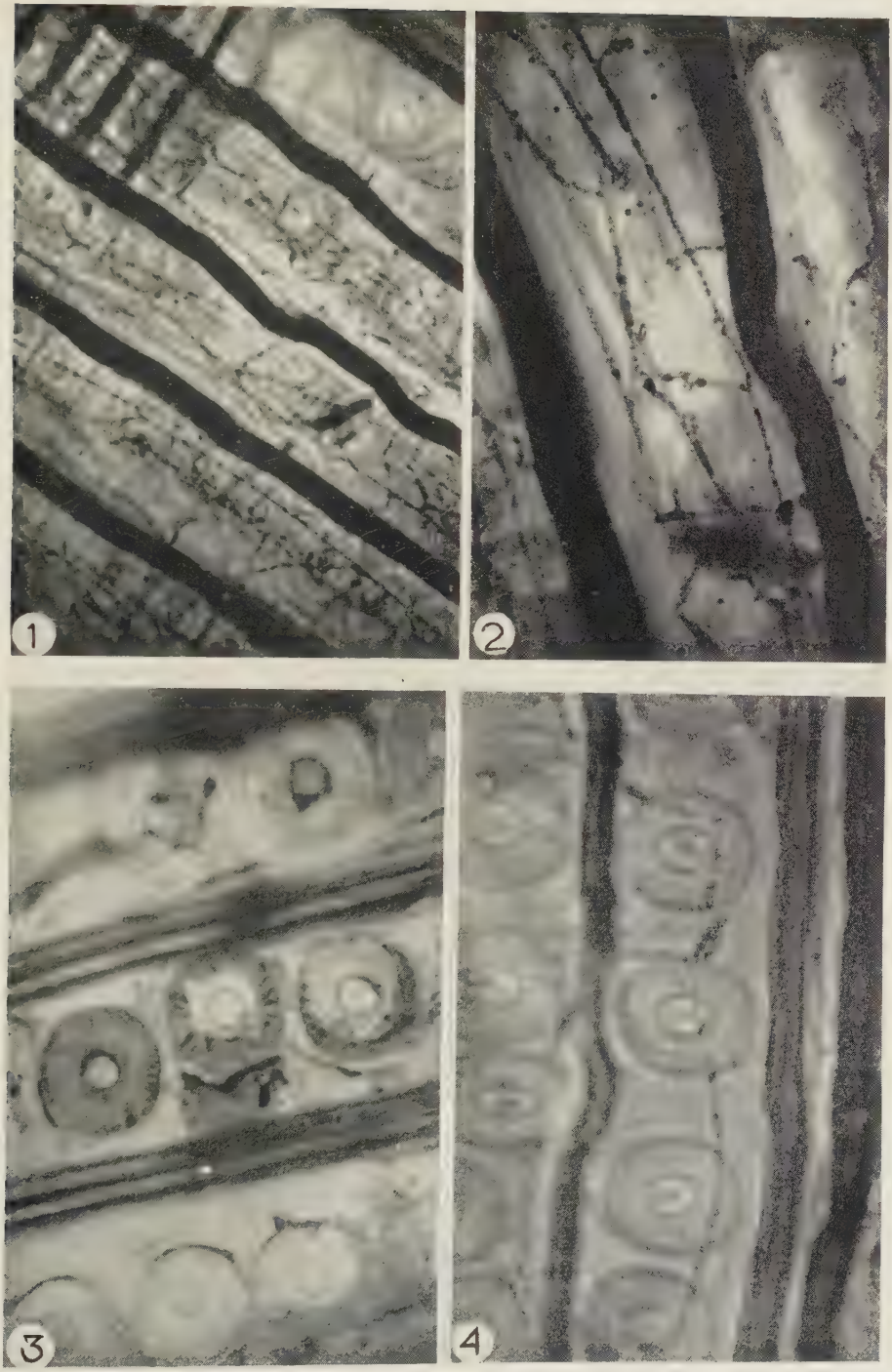
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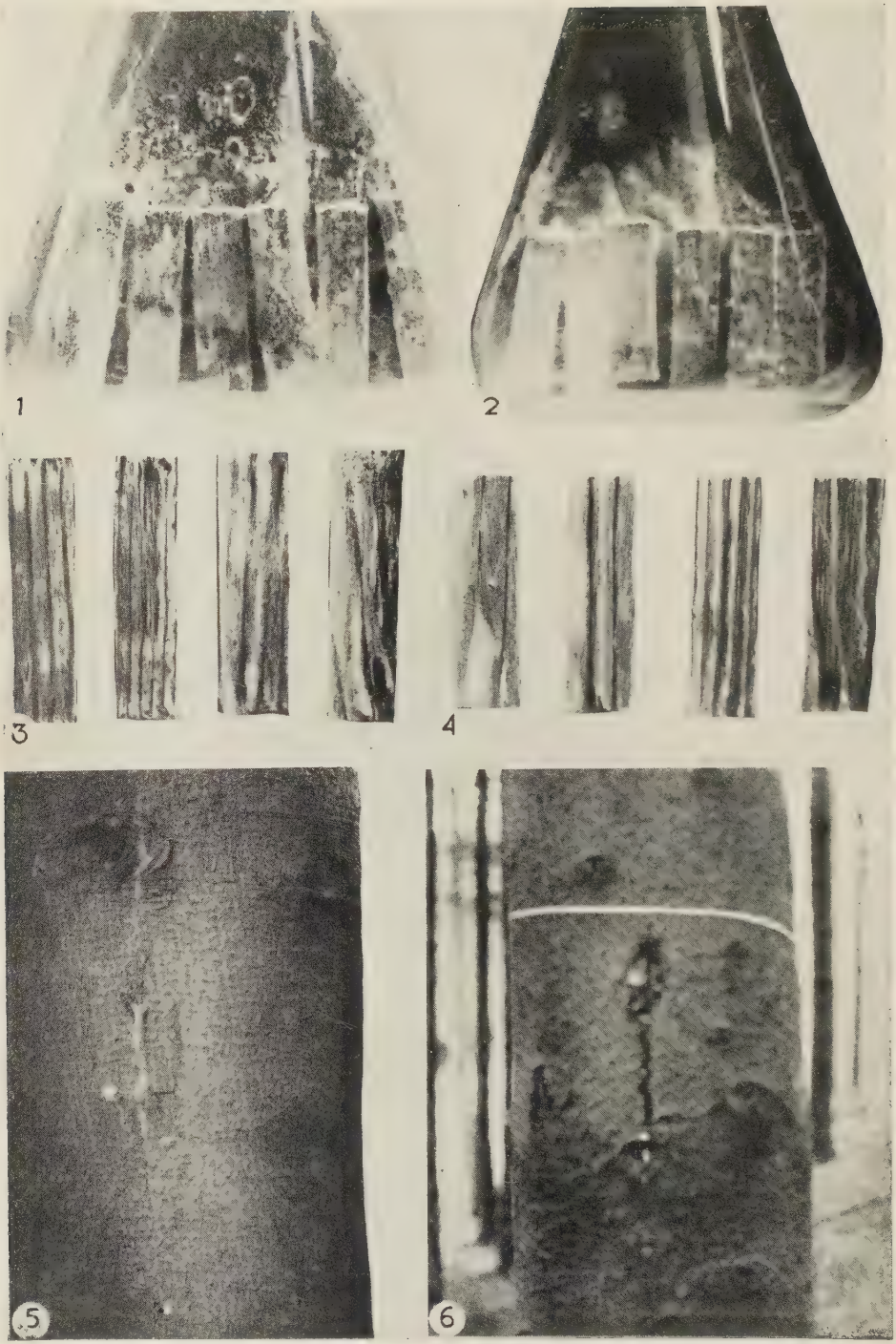

A disease of Norway spruce S. N. Banerjee

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HETEROGENEITY IN THE LABORATORY DATA OF SYMBIOTIC NITROGEN FIXATION

By SATINATH BHADURI

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(With 2 Text-figures)

Abstract

Data on symbiotic nitrogen fixation are generally non-normal owing to different factors both inherent and environmental. Uniformity trials clearly indicate such non-normality of distribution of data on the number of nodules and the dry weight of plants.

In replicated well designed experiments in the laboratory and in the field, environmental factors are controlled; but the pooled estimate of error and consequential tests of significance of treatment effects are invalidated by such inherent non-normality which is often indicated in the correlation between means and variances.

This non-normal distribution can be effectively controlled by the logarithmic transformation of the data and this statistical tool can be successfully used in the laboratory data on symbiotic nitrogen fixation.

Introduction

In recent years replicated experiments and factorial designs have been successfully used in various types of field and laboratory experiments and Fisher's '*t*' and '*z*' tests are especially widely employed. These tests, however, assume the data to be normal or only slightly non-normal; but in strongly non-normal data such tests become invalid. Even when the data are non-normal the comparisons of the treatment differences can be made, provided that the standard deviation is not generally more than 30% of the mean, but if the deviations are of a higher order, non-normality vitiates the results to a great extent.

Experiments on symbiosis between clover plants and nodule bacteria always appear to show great heterogeneity and call for an examination of the distribution of the principal parameters of interest and the application of ordinary statistical methods. In a great variety of different experiments with clover on strain variation and related problems, a large body of data has been accumulated on the number and size of the nodules produced, dry weights of nodulated plants etc. The nodule number, on plants grown in tubes, has been shown to depend upon the concentration of bacteria in the inoculum within limits (Bhaduri, 1951), the volume of the media, rates of sowing and genetical characters of the plants themselves (Nutman, 1946, 1949). In majority of experiments, the type of inoculum, the volume and the nutritive nature of the media and rates of sowing the plants are largely controlled but great heterogeneity remains in the data owing to the practical difficulty in limiting the genetical variability of the plant. Further, heterogeneity results from the interactions of these plant factors with treatment effects. The raising of pure

line of plant material having particular genetical characteristics in unpractical although variability can somewhat be reduced by simple selection. Even when this is done, plant interaction with the bacteria is only partially controlled and the heterogeneity of the data is generally such that the ordinary tests of significance and methods of analysis of the data cannot give reliable and satisfactory information. The following work was undertaken in order to bring out the nature of such difficulties and to suggest means of dealing with them.

Uniformity trials and heterogeneity in experiments on symbiosis

A study of distribution was made utilising a variety of data obtained in experiments with clover plants. Each experiment by itself may be regarded as a uniformity trial, difference between the experiments lying in the age of the plants at which they were harvested and the time of the year during which they were grown. Typical examples of distribution of nodule numbers are presented in Table 1.

Table 1. **k** *and* **ĝ** *statistics of experiments* (Dr. P. S. Nutman's data)

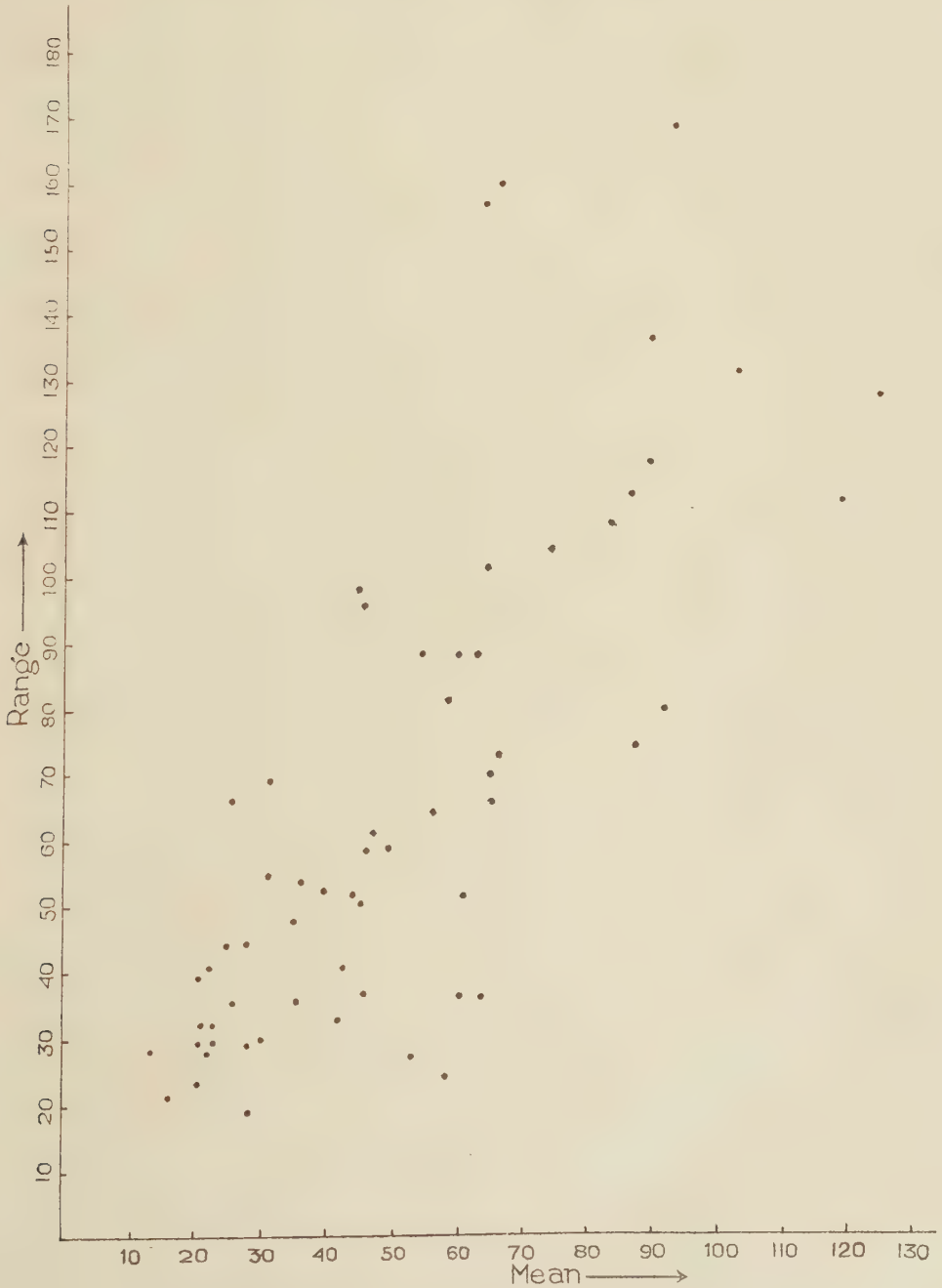
Experiment	D.F.	k-statistics	g-statistics	S.E. of g
I	58	$k_1 =$ 27.4		
		$k_2 =$ 87.0	$g_1 =$ −46.96	±0.098
		$k_3 =$ −38103.5		
		$k_4=$ +21371363.0	$g_2 =$ +7569.00	±0.613
II	61	$k_1 =$ 24.5		
		$k_2 =$ 104.7	$g_1 =$ −15.42	±0.300
		$k_3 =$ −16530.5		
		$k_4 =$ +1206040.0	$g_2 =$ +109.94	±0.190
III	138	$k_1 =$ 23.6		
		$k_2 =$ 128.4	$g_1 =$ +17.33	±0.205
		$k_3 =$ +25214.1		
		$k_4 =$ +1102690.1	$g_2 =$ +168.01	±0.381

Table 1 shows the k-statistics and g-statistics calculated from the data of each experiment according to Fisher (1944). The highly significant g_1 and g_2 values of the experiments I and II shows that the distribution is markedly skew in nature and those of the experiment III kurtotic.

The correlation between standard errors and means of nodule numbers in 60 dissociation experiments are shown by plotting the ranges against the latter (text-fig. 1). In such cases, the analysis of ordinary data by standard methods is meaningless and transformation becomes necessary (Cochran, 1938). It can be shown algebraically that log x's are normally distributed when ranges or standard errors are proportional to the means, and the ordinary methods are available for the analysis of such transformed data. In text-fig. 2, the means and ranges of transformed data of the same dissociation experiments are shown to be quite independent.

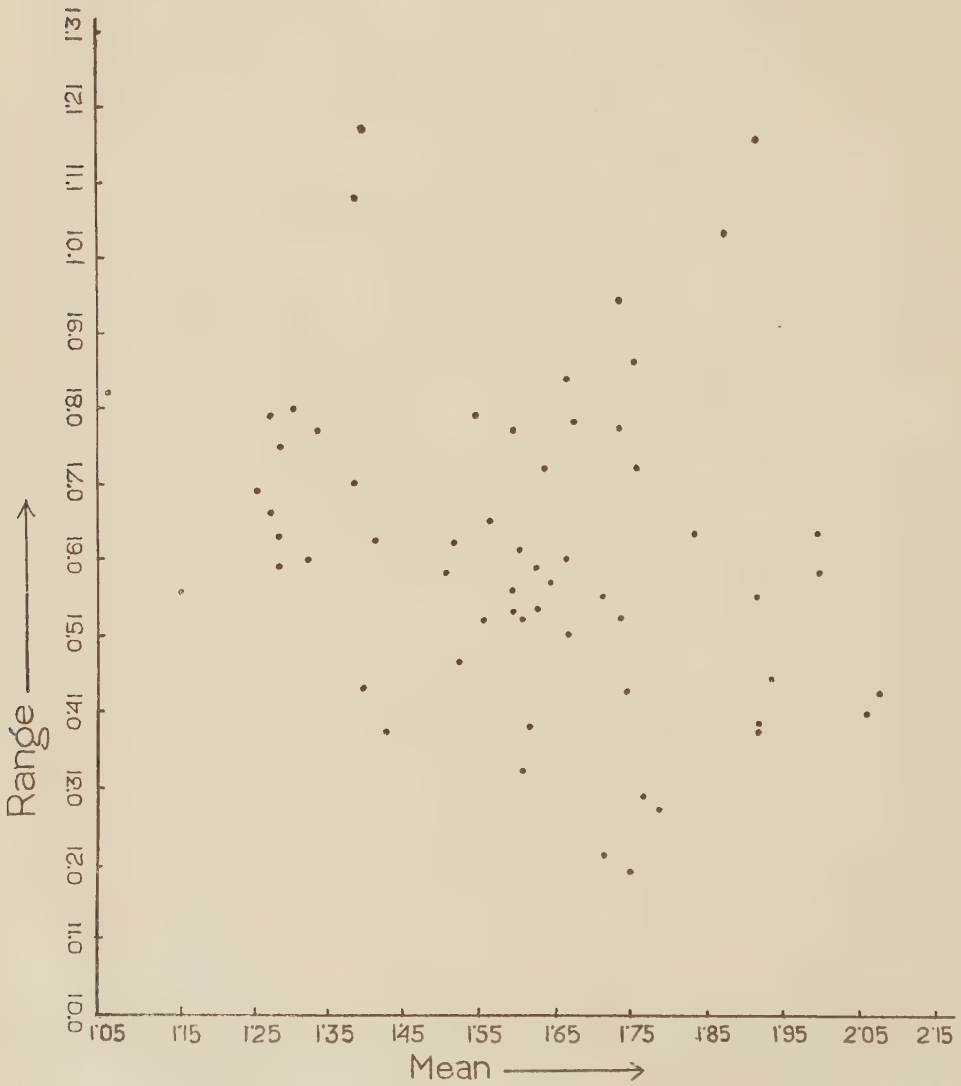
Logarithmic transformation of data has been successfully used in horticultural experiments where different treatments had very large effects

on the diameter of trees (Pearce, 1943). Cochran (1938) has given a summary of methods to be applied when peculiar data accumulates in the laboratory or in the field. Williams (1937) has shown that insect



Text-fig. 1. Diagram showing correlation between means and ranges in the raw data

catches had to be transformed logarithmically as variations in catches were in the order of several thousands from night to night.



Text–fig. 2. Diagram showing the absence of correlation between means and ranges in the log. transformed data

As far as can be ascertained, these methods have not been applied to experiments on symbiosis (Nutman, 1952), though Wilson (1940), when referring to objections that data furnished in experiments on the influence of pN_2 on nitrogen fixation were non-normal, states that similar conclusions can be drawn from logarithmically transformed data.

In comprehensive replicated experiments, the loss of orthogonality arises when one or more samples of the experiment may be lost by unforeseen circumstances, as premature death of the plant, presence of a resistant variety, albinoes, etc. It is very laborious to make necessary analysis after fitting constants to the data owing to the arithmatic involved, and in such cases, orthogonality may be restored by calculating the missing values (Yates, 1933) with necessary changes in the degrees of freedom and standard errors. It may be noted that such missing values should be calculated from the transformed data.

Application of logarithmic transformation to a factorial experiment

The experiment was designed to study the effect of the type of inoculum, influence of the variety of plants and the rate of sowing on the numbers of nodules produced and the effectivity of symbiosis as reflected on the dry weight of plants.

Plants were grown on slopes of agar in test tubes under bacteriologically aseptic condition as described by Thornton (1930). The volume of the medium has been shown to influence considerably on the number of nodules produced (Nutman, 1946), and hence was strictly controlled. The two varieties of clover plants used were '*g*' and '*x*' and the three bacterial strains were A121111, 211 and f12 (Nutman, 1946). The plant variety '*g*' was known to give an effective symbiosis with strain A121111 and 211, and the variety '*x*' which differed essentially from '*g*' only on being homozygous for a recessive gene gave an effective symbiosis with A121111 and ineffective symbiosis with 211. Both varieties, however, gave ineffective symbiosis with f12. The application of missing plot technique became necessary owing to the unavoidable seggregation in this material of plants totally resistant to nodule formation.

The experiment was set up with two varieties in three combinations of spacing viz.—(*i*) a plant growing alone, (*ii*) a plant growing in the presence of one of its own vareity and (*iii*) a plant growing in presence of the other variety, and when inoculated with each of the three strains. The whole scheme of treatments in this $2 \times 3 \times 3$ factorial design is summarised in Table 2.

Table 2. *Symbols used for different treatments*

Strains	Plant alone		Plant with own variety		Plant with the other variety	
	g	x	gg	xx	Gx	gX
A121111	Ag	Ax	Agg	Axx	AGx	AgX
211	Bg	Bx	Bgg	Bxx	BGx	BgX
f12	Cg	Cx	Cgg	Cxx	CGx	CgX

N.B.—A, B and C stand for the strains A121111, 211 and f12 respectively.

Instead of making two sets of tubes, for the last two columns of Table 2, with the same plants growing under identical conditions, it was worth while making only one set of tubes and counting the nodules of both '*g*' and '*x*' plants from the same tube, thus affecting a considerable reduction in the bulk of the experiment. In such a case a serious loss of efficiency is liable to occur, if there were a correlation between the symbiotic effects of the two plants in the same tube. No such correlation was, however, evident in the data.

Externally sterilised seeds were grown in test tubes containing 10 cc. of nitrogen defficient nutrient agar (Bhaduri, 1951) and were inoculated with bacterial strains when the plants were 21-days-old. Each test tube was supplied with 2 cc. of appropriate inoculum standardised by haemocytometer count to contain the same number of bacteria per cc. The water level was marked on the test tube and the volume of the liquid in the test tube was kept constant by adding requisite amount of sterile water periodically. Eight replicate tubes of each treatment were made and randomised in blocks. Plants were grown for three months after which nodule counts were made and dry weights of plants were determined.

The particular interest in this experiment lay in the study of the influence of spacing, plant vareity and the types of strains on the interaction which occurs between plants occupying the same tube.

On harvesting, it was found that as many as 6 tubes with '*x*' plants were without nodules in the whole experiment owing to plants being resistant. When such resistant plants were present in tubes containing both '*g*' and '*x*' plants, numbers of nodules and dry weights of both plants were calculated by the missing plot technique (Yates, 1933).

Table 3. *Comparison of different transformations of* nodule number *and* dry weight *data*

	Nodule numbers			Dry weights	
	Raw data	Square root transformation	Log. transformation	Raw data	Log. transformation
Replicate variance (7 d.f.)	1476.03*	4.797*	0.06817*	205.19**	0.0427*
Treatment variance (17 d.f.)	2764.79***	11.384***	0.14754***	1246.11***	0.3562***
Error variance (113 d.f.)	531.98	2.109	0.03177	72.15	0.01567
General mean	64.05	7.801	1.7591	24.35	1.3181
S.E. as percentage of the mean	±36.01%	±18.62%	±10.13%	±34.88%	±9.50%
Z, for Replicate variance (n_1 = 7 d.f.) (n_2 = 113 d.f.)	0.50942	0.41099	0.39163	0.52190	0.50215
Z, for Treatment variance (n_1 = 17 d.f.) (n_2 = 113 d.f.)	0.82433	0.84302	0.76779	1.4245	1.5394

* asterisk indicates 5% point of significance.
** and *** asterisks indicate 1% and 0.1% points of significance.

Table 4. *Computation of χ^2 of data on nodule number per plant*

No.	Treatment	Sum of Squares	D.F. n	$\frac{1}{n}$	Variance v	log. v
1	BgX	932.875	7	0.1429	133.3	2.1248
2	AgX	2094.875	7	,,	299.3	2.4761
3	Axx	2804.720	7	,,	400.7	2.6028
4	Bxx	1579.220	7	,,	225.6	2.3533
5	Cxx	4124.500	7	,,	574.9	2.7596
6	Bx	3988.000	6	0.1667	664.7	2.8226
7	Cx	1239.200	4	0.2000	309.8	2.4911
8	Ax	4850.520	6	0.1667	808.4	2.9076
9	CgX	7432.860	6	,,	1061.8	3.0258
10	Agg	879.470	7	0.1429	125.6	2.0990
11	Bgg	1010.875	7	,,	144.4	2.1596
12	BGx	2867.875	7	,,	409.7	2.6125
13	AGx	6156.000	7	,,	879.4	2.9442
14	Cgg	4523.375	7	,,	648.2	2.8117
15	Ag	4694.500	7	,,	670.6	2.8265
16	Bg	6662.875	7	,,	951.8	2.9785
17	CGx	4628.875	7	,,	661.3	2.8204
18	Cg	10281.500	7	,,	1464.8	3.1658
	Total	70752.120	120	—	—	—

(*N.B.*—For expalnation of treatment symbols see Table II).

$S(n.\ \log\ v) = 319.6440$; $S^2 = \frac{70752.120}{120} = 589.6$; $n.\log s^2 = 332.4720$;

$\therefore\ \chi^2 = 2.3020\ (n.\log s^2 - S(n.\log v)) = 29.54$, with 17 degrees of freedom

Corrected $\chi^2 = 28.05$; this is significant with a p between 0.05 and 0.02.

The analysis of variance of nodule numbers and dry weights of plants in Table 3 show the standard errors as high as 36.01% and 33.88% of the general mean respectively. Such high percentages of standard errors occur in non-normal data. Eighteen different treatment variances have also been tested for homogeneity by the method evolved by Bartlett (1936). In the computations shown in Table 4 the corrected $\chi^2 = 28.05$ for 17 degrees of freedom is clearly significant so that there are real differences between the variances of the treatments and as such the comparison of means with pooled estimate of error is incorrect. Although the Standard Error of nodule numbers is reduced to 18.62% by square root transformation, yet the correlation between means and variances is evident. The correlation is, however, completely eliminated by the logarithmic transformation and the pooled estimate of error, then, can be used successfully for testing treatment effects.

Acknowledgments

The author is indebted to Dr. H. G. Thornton, F.R.S. and Dr. P. S. Nutman for valuable criticism and encouragement during the work done at Rothamsted Experimental Station, England.

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EFFECT OF AGE AND STORAGE CONDITIONS ON THE SUSCEPTIBILITY OF CERTAIN VEGETABLES TO ATTACK BY TISSUE-ROTTING FUNGI.

By SUDHANSU BHUSAN CHATTOPADHYAY

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(With 3 Text-figures)

Abstract

This investigation has been undertaken to study the basis of resistance shown by the average host to the attack of the average fungus, exemplified by the behaviour of *Botrytis cinerea* to living potato tissue. For this purpose, susceptibility of certain vegetable tissues, brought to a definite physiological condition, to the attack of a number of tissue-rotting fungi has been studied.

Pre-storage at 30°C. or 35°C. (as compared with 15°C.) increases the susceptibility of certain plant tissues (potato, swede, turnip, carrot) to invasion by a number of fungi, whether normally parasitic upon these hosts or not. The effect increases with length of the pre-storage period, and is greater at 35°C. than at 30°C.

Susceptibility of potato tubers varies with their maturity. Young tubers are susceptible to attack by certain fungi which are classed as non-pathogenic to potato; as the tubers mature, susceptibility decreases and reaches a very low value; at the time of dormancy it then increases with sprouting. With *Pythium de baryanum* and *Phytophthora erythroseptica* which are normal parasites of potato, susceptibility is low with young tubers and increases continuously with advancing maturity and finally with sprouting. When dormant tubers are caused to sprout by artificial means, susceptibility is increased.

Heat pre-treatment beyond a certain time has a damaging effect on tissue, as determined by the plasmolytic method. The safety limits were determined for the tissues used. Heat pre-treatment increases the absorbing capacity of the cells, has no effect on *p*H of the cell-sap and brings about change in the cell-wall substance which are reflected in its staining reactions and in the greater readiness with which it is decomposed by fungal enzymes. Heat pre-treatment of tissue increases the exosmosis of salts and sugars from the cells. This result was demonstrated by physical and chemical methods as well as by the effect of the diffusates in stimulating fungal germinations, growth and enzyme secretion.

The effect of maturity on susceptibility is not due to cell-wall changes but is closely correlated with exosmosis of nutrients.

The specificity of some of the fungi used to tissues is due in part at least to reactions arising from the nature of the plant sap. These may interfere with growth of the fungus, with secretion of the pectinase enzyme or with the functioning of the latter. Illustrations of these effects are given.

The amount of rotting brought about by a small quantity of fungal enzyme, when applied to a relatively large piece of potato tissue, is not correlated with the readiness with which the enzymic liquid is absorbed by the sub-turgid tissue. It is determined by the concentration of the enzyme present, and by the source of the latter. *Botrytis* enzyme is less effective than *Pythium* enzyme of the same strength, as determined by the time required to macerate discs of tissue of standard thickness.

Introduction

The nature of host resistance to the attack of a parasite has always been a subject of interest in plant pathology, either from the academic

or the practical point of view. The internal resistance offered by the host to the attack of an invading organism generally falls into two categories—mechanical and chemical. The former is concerned either with the various properties of the cell-wall e.g., structure, composition, thickness, or the capacity of the cell to develop some barrier in the form of cork cells or gum in advance of the pathogen. The chemical factors that are responsible are usually the acidity of the cell-sap or the presence of certain toxic substances in the cell such as tannins or glucosides.

As pointed out by Brown (1934, 1936) a more general and widely occurring type of resistance is that shown by the average host to the attack of the average fungus. In such cases, the host tissue may have nothing inhibitory or harmful to the growth of the fungus and the fungus also may not require any special food for its growth or enzyme secretion. Nevertheless, attack does not occur even though the necessary conditions seem to be present. A familiar example of this is the behaviour of *Botrytis cinerea* in relation to living potato tissue. *Botrytis cinerea* can grow well on potato decoctions and on dead potato tissue, and is capable of secreting the enzyme pectinase under those conditions, but under normal conditions there is no attack.

The present investigation has been undertaken with a view to determine more closely the basis of resistance of the host tissue in such cases of general nature. Certain vegetable tissues have been chosen for this work, as being readily available and amenable to experimental treatments. After certain treatments which were devised so as to bring these tissues into a certain physiological condition, they were tested in a systematic manner with regard to their resistance to invasion by certain fungi. With such quantitative data available, it was hoped that a closer insight would be obtained into the mechanism of resistance on the one hand and the nature of the aggressive apparatus of the fungus on the other.

Historical

Effect of storage at high temperature prior to inoculation

Vasudeva (1930) first observed that apples of the Newton variety, when artificially "ripened" by storage at 30°C. for several days, were attacked by *Botrytis allii* while the control ones kept at laboratory temperature were not. He determined the acidity, sugar and nitrogen contents of the extract of apples after storage at the temperatures stated but was unable to explain the changed susceptibility to attack on the basis of changes in any of these factors.

A similar result was obtained by Chona (1932) who noted that apples of the Newton variety after storage at 35°C. for 7 days became more susceptible to attack of two strains of *Fusarium fructigenum*.

Later, Fernando (1934) found that *Bacillus subtilis*, which is normally a saprophyte, can attack potato tubers of the Epicure variety if they are stored previously at 38–40°C. for several days.

Effect of Age

In apple, Horne (1930), Vasudeva (1930) and Chona (1932) found diminishing resistance to attack with gradual ripening. Horne (1930) considered that this was due to progressive diminution of the acidity of the cell-sap and to increase in the soluble nitrogen content with advancing age.

Chona (1932) found that Kerr's Pink variety of potato become more susceptible to the attack of *Fusarium caeruleum* with progressing age and maturity. This increase in susceptibility to attack with maturity had been observed by Pethybridge and Lafferty (1927) and later by Small (1944). Small (l.c.) also observed that young immature tubers are usually not susceptible to the attack of *F. caeruleum* but that there were exceptions to this rule.

Material and methods

Fungi and host tissues used

In the present investigation, the following fungi were used :—(1) three different strains of *Botrytis cinerea* Pers., namely, (*a*) *Lettuce strain* (from stem lesion), (*b*) *Daffodil strain* (from diseased bulb) and (*c*) *Gladiolus strain* (from diseased corm); (2) *Fusarium fructigenum* Fries. ("A" strain of Brown—from apple fruit); (3) *Rhizopus nigricans* Ehr.; (4) *Pythium de baryanum* Hesse (from a rotted potato); (5) *Phytophthora erythroseptica* Peth. (from diseased potato tuber) and (6) Two different strains of *Rhizoctonia solani* Kuhn., namely, (*a*) Potato strain (from diseased sprout) and (*b*) Crucifer strain (from diseased seakale leaf).

All these fungi were obtained from stock cultures kept at the Plant Pathology Laboratory, Imperial College of Science and Technology. The cultures of *Fusarium fructigenum*, *Rhizopus nigricans* and the three strains of *Botrytis cinerea* were maintained on slopes of potato agar, the two strains of *Rhizoctonia solani* on malt agar and *Pythium de baryanum* and *Phytophthora erythroseptica* on oatmeal agar.

In those experiments where only one strain of *Botrytis cinerea* was used, the lettuce strain was always chosen.

Two varieties of potato tubers, namely King Edward and Majestic, were chiefly used in this work. The other varieties used from time to time were Epicure, Sharpe's Express, Arran Pilot, Great Scot, Arran Banner, and Up-to-date. The other vegetables studied were swede and carrot.

Method of inoculation

As a routine measure, spores required for inoculations were taken from 10–15 days old cultures and mycelial pieces from 4–6 days old cultures. To eliminate contaminants, potato tubers and swede roots were first washed in water, then dipped for 10 minutes in 0.2% solution of mercuric chloride and finally washed with water. Potato tubers and root vegetables were inoculated by introducing the fungus into the host tissue after the method of Granger and Horne (1924). This was standardised by inserting the cork borer (No. 2 size) to a definite depth (1.5 cm.). After inoculation,

the vegetables were stored in large sterile tin boxes. There was no necessity for any arrangement to maintain humidity inside the box as the water lost by the vegetables during storage was sufficient to maintain a moist atmosphere inside the box. In all cases, the inoculated material was incubated at 20°C. With potato tubers and root vegetables, the amount of attack was determined by weighing the quantity of rotted tissue after 7 days inoculation. In each determination of the amount of attack, ten replicates were always used.

Preparation of pectinase

To obtain the enzymic preparation of *Botrytis cinerea*, Brown's plate technique (1915) was employed. The dried mycelium after being powdered with an equal quantity of sand, was used for obtaining the enzyme. A watery suspension of enzyme was prepared by digesting the powder with water, the usual quantity being 0.2 gm. of powder in 3 c.c. of water.

To prepare the enzyme of *Pythium de baryanum*, Menon's (1934) method was mainly used.

An alternative method which gave a stronger enzymic solution from *Pythium de baryanum* was to grow the fungus in a flask containing 10 c.c. of the following medium : Asparagin 4.5 gm., Glucose 5.0 gm., Peptone 4.5 gm., $MgSO_4$ 1.6 gm., K_3PO_4 3.3 gm., Starch 25.0 gm. and Water 1,000 c.c. After 10 days' growth at 20°C., the liquid was used as such as a source of enzyme.

Determination of enzymic activity

This was done according to Brown's method (1915) by noting the time taken for the loss of coherence of discs of potato (1.5 cm. in diameter and 0.5 mm. in thickness) when they are immersed in enzymic solutions. In such tests, 3 discs were placed in 3 c.c. of the liquid.

Effect of pre-storage temperature on amount of attack

(a) *Potato*

The main experiments under this heading were carried out with a large batch of tubers of the varieties King Edward and Majestic. In order to secure as much uniformity as possible, tubers of approximately equal size were sorted out, and half of each batch stored at 15°C. and the other half at 35°C. At intervals, tubers were taken out, inoculated with the test fungi and then stored at 20°C. At the end of 7 days incubation (at 20°C.), the amount of rotted tissue was weighed in each case. For each determination of rotting due to each fungus, 10 tubers were used. Each figure in the Tables (1–2) thus represents the mean of 10 determinations. This series of experiments was carried out from January to early March, i.e., at the period when the tubers are dormant and unsprouted (in England). Of the fungi used, only three, viz., *Pythium de baryanum*, *Phytophthora erythroseptica* and *Rhizoctonia solani* (potato) are normally associated with potato and thus are able to produce a material amount of rotting

Table 1. *Effect of pre-storage at 35°C. and 15°C on susceptibility of* King Edward potato

Temp. of pre-storage	Period of storage (days)	*Botrytis cinerea* (lettuce)	*Botrytis cinerea* (daffodil)	*Botrytis cinerea* (gladiolus)	*Fusarium fructigen*	*Rhizopus nigricans*	*Pythium de baryanum*	*Phytophthora ergroseptica*	*Rhizoctonia solani* (potato)	*Rhizoctonia solani* (cruciferr)
	1	0.027± 0.015	0.021± 0.012	0.069± 0.017	0.164± 0.014	0.084± 0.034	0.462± 0.057	3.380± 0.33	0.566± 0.066	0.072± 0.022
	2	0.148± 0.029	0.118± 0.023	0.305± 0.048	0.209± 0.042	0.134± 0.036	0.896± 0.046	4.574± 0.411	0.655± 0.016	0.078± 0.014
35°C	3	0.256± 0.022	0.266± 0.031	0.442± 0.038	0.388± 0.03	0.286± 0.01	1.195± 0.03	5.550± 0.34	0.733± 0.12	0.153± 0.024
	5	0.541± 0.053	0.355± 0.015	0.501± 0.033	0.415± 0.026	0.406± 0.028	1.316± 0.12	7.846± 0.48	0.915± 0.13	0.320± 0.019
	7	0.563± 0.025	0.465± 0.036	0.582± 0.027	0.555± 0.04	0.472± 0.035	2.121± 0.28	7.919± 0.51	1.073± 0.12	0.366± 0.021
	10	0.565± 0.035	0.475± 0.035	0.606± 0.026	0.713± 0.033	0.542± 0.032	2.634± 0.19	8.70± 1.33	1.10± 0.13	0.402± 0.012
	15	0.983± 0.075	0.881± 0.084	0.931± 0.083	0.887± 0.073	0.800± 0.057	7.312± 0.142	11.54± 1.49	1.481± 0.13	0.535± 0.022
	1	0.026± 0.012	0.022± 0.012	0.065± 0.023	0.154± 0.02	0.074± 0.02	0.463± 0.024	3.380± 0.33	0.522± 0.07	0.066± 0.027
	2	0.025± 0.009	0.021± 0.008	0.062± 0.017	0.148± 0.012	0.060± 0.036	0.484± 0.028	3.320± 0.41	0.520± 0.025	0.070± 0.023
15°C	3	0.022± 0.007	0.025± 0.008	0.058± 0.019	0.162± 0.034	0.068± 0.021	0.500± 0.059	3.150± 0.32	0.511± 0.033	0.070± 0.019
	5	0.032± 0.014	0.023± 0.007	0.056± 0.007	0.158± 0.028	0.071± 0.018	0.520± 0.036	3.168± 0.28	0.522± 0.038	0.072± 0.012
	7	0.030± 0.015	0.022± 0.005	0.051± 0.009	0.176± 0.031	0.066± 0.025	0.514± 0.073	3.364± 0.49	0.518± 0.039	0.080± 0.032
	10	0.031± 0.009	0.022± 0.008	0.056± 0.01	0.170± 0.029	0.076± 0.013	0.501± 0.041	3.170± 0.51	0.524± 0.063	0.084± 0.012
	15	0.026± 0.009	0.023± 0.008	0.060± 0.007	0.163± 0.025	0.070± 0.020	0.480± 0.075	3.522± 0.44	0.519± 0.039	0.071± 0.012

Table 2. *Effect of pre-storage at 35°C. and 15°C. on susceptibility of* Majestic potato

Temp. of pre-storage	Period of storage (days)	*B. cinerea* (lettuce)	*B. cinerea* (daffodil)	*B. cinerea* (gladiolus)	*F. fructigenum*	*R. nigricans*	*P. de baryanum*	*P. erythroseptica*	*R. solani* (potato)	*R. solani* (crucifer)
35°C	1	0.084± 0.022	0.056± 0.02	0.133± 0.028	0.221± 0.024	0.128± 0.02	0.566± 0.072	3.50± 0.59	0.520± 0.01	0.081± 0.028
	2	0.211± 0.023	0.168± 0.025	0.211± 0.033	0.335± 0.018	0.260± 0.032	0.924± 0.062	4.348± 0.18	0.634± 0.024	0.139± 0.015
	3	0.519± 0.067	0.531± 0.059	0.533± 0.06	0.432± 0.012	0.298± 0.03	0.967± 0.092	5.931± 0.20	0.680± 0.034	0.208± 0.042
	5	0.526± 0.056	0.547± 0.043	0.616± 0.046	0.619± 0.02	0.478± 0.028	1.563± 0.296	8.120± 1.28	0.734± 0.029	0.236± 0.041
	7	0.546± 0.061	0.547± 0.033	0.632± 0.014	0.750± 0.04	0.590± 0.026	1.620± 0.18	9.293± 1.12	1.105± 0.06	0.346± 0.027
	10	0.652± 0.051	0.555± 0.029	0.671± 0.073	0.827± 0.03	0.622± 0.09	2.013± 0.31	10.405± 1.05	1.173± 0.101	0.398± 0.054
	15	1.353± 0.14	1.161± 0.22	1.553± 0.233	0.950± 0.048	0.903± 0.033	8.231± 0.88	14.260± 2.011	1.570± 0.11	0.521± 0.023
15°C	1	0.078± 0.029	0.065± 0.016	0.114± 0.02	0.196± 0.01	0.114± 0.028	0.562± 0.06	3.390± 0.43	0.501± 0.044	0.080± 0.023
	2	0.092± 0.022	0.069± 0.016	0.112± 0.026	0.214± 0.013	0.125± 0.013	0.560± 0.037	3.714± 0.64	0.518± 0.032	0.074± 0.036
	3	0.101± 0.018	0.074± 0.011	0.126± 0.017	0.026± 0.12	0.110± 0.011	0.582± 0.038	3.630± 0.57	0.510± 0.032	0.071± 0.013
	5	0.070± 0.014	0.070± 0.014	0.120± 0.031	0.226± 0.025	0.112± 0.033	0.548± 0.072	3.590± 0.27	0.524± 0.071	0.086± 0.02
	7	0.085± 0.017	0.080± 0.019	0.122± 0.028	0.214± 0.02	0.110± 0.019	0.522± 0.041	3.384± 0.40	0.499± 0.025	0.081± 0.027
	10	0.097± 0.023	0.083± 0.021	0.137± 0.023	0.206± 0.023	0.128± 0.025	0.582± 0.053	3.756± 0.31	0.520± 0.04	0.073± 0.017
	15	0.081± 0.029	0.068± 0.018	0.123± 0.019	0.199± 0.014	0.126± 0.024	0.567± 0.037	3.413± 0.26	0.523± 0.05	0.072± 0.009

under ordinary circumstances. The remainder are normally not parasitic upon potato tubers. In the case of *Botrytis cinerea* (3 strains) and *Fusarium fructigenum*, inoculations were made as uniformly as possible by adding 0.1 c.c. of a spore suspension; with others equal sized pieces of mycelium were used. The various particulars of the experiments are set out in the headings to Tables 1–2. The figures in these Tables give the mean amount (grams) of rotted tissue together with the standard error of each mean.

The analysis of variance has been worked out to show the significance of the effect of various factors, namely, variety, fungus, temperature, period and their interactions. The analysis has been carried out by substituting for each original figure (x) the quantity $\log_{10} 100x$. The reason is that the weight of rotted tissue is a function of the amount of fungal growth which increases logarithmically. Under such circumstances, i.e., logarithmic growth, the variance increases with the square of the mean. In order to obtain a normally distributed error, it is therefore necessary to transform the data to a logarithmic scale. Another reason for this procedure is that the difference between smallest and largest figures in the Tables is very great, viz., 500 times. In such cases logarithmic transformation is necessary to bring the figures within comparable limits. The use of the logarithmic scale as pointed out by Fisher and Mackenzie (1923) and Cochran (1938) has also the advantage that it provides a better test of independence or interaction of treatments.

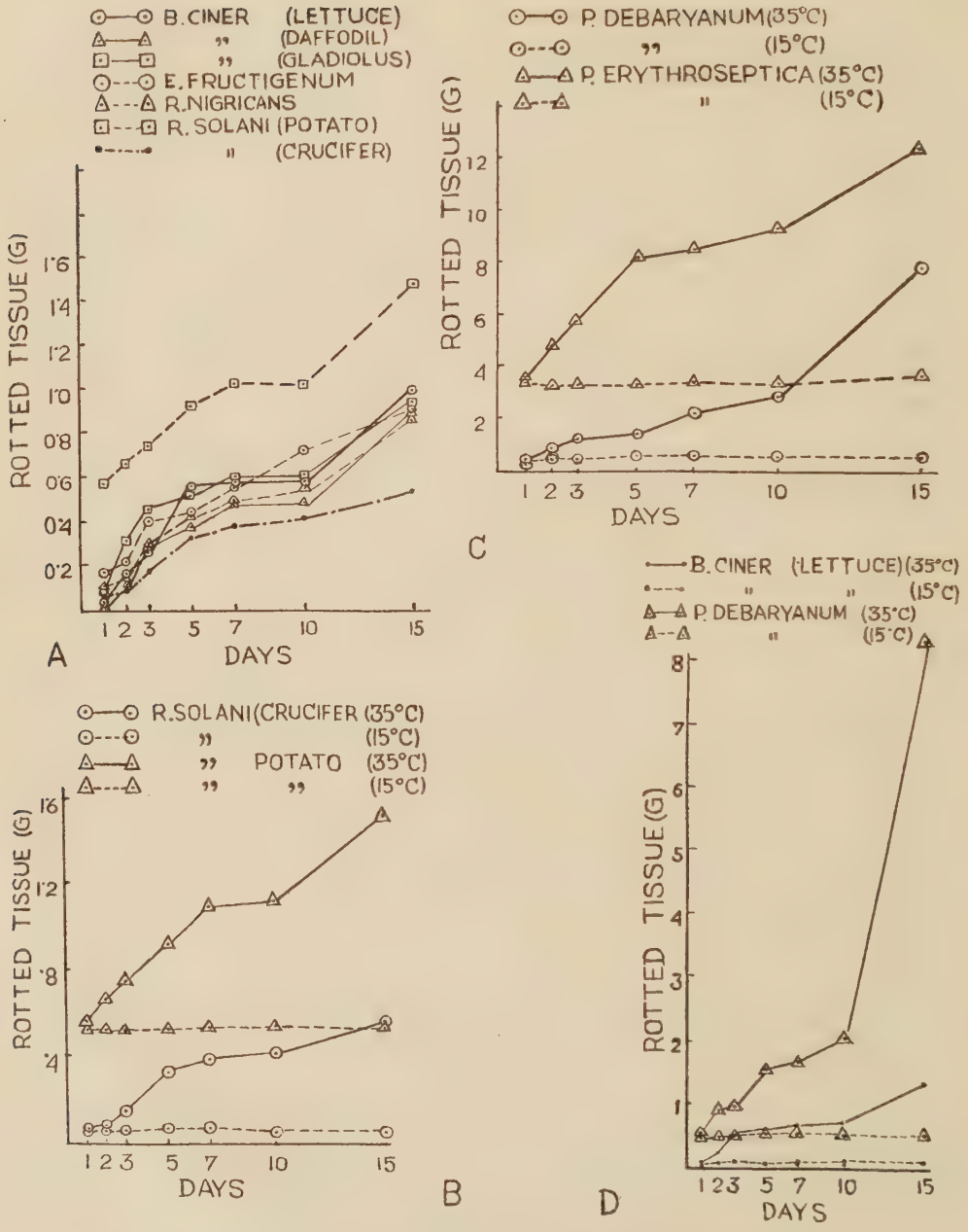
Where significant results are obtained, significant differences applicable to the mean of the logarithmic data have been calculated from analysis of variance.

Table 3. *Analysis of variance* of data in Tables 1–2

Due to	Degrees of Freedom	Variance Ratio (F)	Probablity
Pre-storage Temp.	1	1617.9	$P<0.01$
Period of Storage	6	10.54	,, ,,
Fungus	8	701.89	,, ,,
Variety	1	99.99	,, ,,
Pre-storage Temp. × Period	6	73.61	,, ,,
Pre-storage Temp. × Fungus	8	31.62	,, ,,
Pre-storage Temp. × Variety	1	21.83	,, ,,
Fungus × Variety	8	13.38	,, ,,
Fungus × Period	48	1.44	Not Sig.
Variety × Period	6	0.99	,, ,,
Pre-storage Temp. × Period × Fungus	48	1.00	,, ,,
Pre-storage Temp. × Fungus × Variety	8	0.48	,, ,,
Variety × Fungus × Period	48	0.184	,, ,,
Variety × Pre-storage Temp. × Period	6	0.041	,, ,,
Error	48		

The general results indicated in the Tables 1–2 and the statistical analysis of these may be summarised as follows :—

Pre-storage at 35°C. increases susceptibility of the tubers of the two varieties tested to rotting by the fungi studied. The susceptibility increases



Text-fig. 1. A, Effect of length of pre-storage at 35°C on amount of rotted tissue produced by different fungi on king Edward potato. B, Effect of length of prestorage at 35°C and 15°C on amount of rotted tissue produced by *P. de baryanum* and *P. erythroseptica* on King Edward potato. C, as in B by two strains of *R. solani* (potato and crucifer). D, Effect of length of pre-storage at 35°C and 15°C on amount of rotted tissue produced by *B, cinerea* and *P. de barynum* on Majestic potato.

with the duration of the pre-storage period. Tubers kept at 15°C. show practically the same susceptibility at all stages of the experiment and the constancy of the amount of attack shown on these by each fungus indicates that there was no change in the parasitic vigour of each fungus, as taken from various subcultures during the course of the experiment. The results given by the tubers stored at 15°C. constitute in fact the controls to those which were kept at 35°C.

The increased susceptibility arising from pre-storage at 35°C. is significant within 3 days or less for all the fungi except the two strains of *Rhizoctonia solani*. With these, difference becomes significant after 5 days. From the beginning of the pre-storage period until about the 5th day, increase of susceptibility is roughly proportional to duration of storage; from the 5th day to the 10th day, the rate of increase of susceptibility with time of storage is more gradual, but is followed by a rapid increase from the 10th day to the 15th day.

The fungi differ from each other in the absolute amount of rotting produced, but the same general tendency to increased attack on tubers pre-stored at 35°C. is shown by all. *Botrytis cinerea* (3 strains), *Fusarium fructigenum*, *Rhizopus nigricans*, and *Rhizoctonia solani* (crucifer) which behave as weak parasites under normal conditions, produce definite and substantial rotting when the tubers are kept previously at 35°C. for some time. In the case of *Pythium de baryanum*, *Phytophthora erythroseptica* and *Rhizoctonia solani* (potato), the attack is much augmented when the tubers are stored at 35°C.

There is a significant difference between the two varieties in the amount of attack under comparable conditions, Majestic being the more susceptible, both for tubers stored at 15°C. and at 35°C. This result applies, in particular, to the five fungi namely, *Botrytis cinerea* (3 strains), *Fusarium fructigenum* and *Rhizopus nigricans*. The greater susceptibility of Majestic shown in the experiments in Tables 1–2 was not always in evidence in comparisons done at other times. The effect shown in this case is therefore probably an accidental one, depending perhaps on the stock of tubers used at different times.

(b) *Swede*

Swedes were stored at 35°C., 30°C. and 15°C., and batches of 10 inoculated after the manner already described for potato. With this vegetable, the storage period at 35°C. could not be extended beyond 7 days on account of damage to the tissue arising from longer exposures. The experiment was conducted with four fungi, viz., *Botrytis cinerea* (Lettuce), *Fusarium fructigenum*, *Phytophthora erythroseptica* and *Rihizoctonia solani* (crucifer).

The Table 4 shows that results for swede follow the same general trend as for potato, viz., an increase in susceptibility which increases with duration of pre-storage, and which is greater the higher the temperature. It will be noted, however, that two of the fungi used, *Pythium de baryanum* and *Rhizoctonia solani* (potato strain), failed to respond to the heat pre-treatment. A statistically significant increase of susceptibility

was obtained by the 3rd day for *Botrytis cinerea* (lettuce strain), *Phytophthora erythroseptica* and *Rhizoctonia solani* (crucifer strain) and by the 5th day for *Fusarium fructigenum.*

Table 4. *Effect of pre-storage temperature on susceptibility of* swede.— *Analysis of variance*

Due to	Degrees of Freedom	Variance Ratio (F)	Probablity
Period of Storage	3	23.40	P<0.01
Fungus	3	528.37	,,
Pre-storage Temp.	2	55.96	,,
Period × Pre-storage Temp.	6	5.68	,,
Period × Fungus	9	0.16	Not sig.
Pre-storage Temp. × Fungus	6	0.32	,,
Error	18	—	

It was noted that the order of pathogenicity on swede of the four organisms is *B. cinerea, P. erythroseptica, R. Solani* (crucifer) and *F. fructigenum* in diminishing series. The two last produce a more or less dry type of rot in contrast to the wet rot produced by the first two.

(c) *Carrot*

The results obtained for carrot in experiments conducted along the same lines as described are assembled in Table 5. With this host it was not practicable to extend the heat treatment at 35°C. beyond 5 days as longer exposure led to breakdown of the tissue and rotting by miscellaneous organisms. Of the fungi used, viz., *Botrytis cinerea, Rhizopus nigricans, Fusarium fructigenum, Phythium de baryanum, Phytophthora erythroseptica* and 2 strains of *Rhizoctonia solani,* all but the first two failed to produce attack under any conditions.

Table 5. *Effect of pre-storage at 35°C., 30°C. and 15°C. on susceptibility of* carrot

Pre-storage Temp.	Days	*B. cinerea* (Lettuce)	*R. nigricans*
35°C	1	2.195±0.20	2.320±0.14
	3	3.197±0.26	all rotted
	5	7.364±0.17	all rotted
	7	spoiled	spoiled
30°C	1	1.726±0.12	1.798±0.15
	3	2.706±0.34	3.562±0.36
	5	4.252±0.28	6.05 ±0.32
	7	5.420±0.35	14.31 ±1.12
15°C	1	1.440±0.14	1.19 ±0.11
	3	1.320±0.21	1.23 ±0.19
	5	1.375±0.11	1.29 ±0.26
	7	1.480±0.24	1.25 ±0.16

To determine whether there is any significant difference between mean of the controls (those kept at 15°C.) and treated (those kept at 30°C. and

35°C.) and also between the mean of different periods in the same temperature, Fisher's '*t*' test (1944) was applied.

In assessing the data of Table 7, it will be safe not to stress the effects produced by exposure to 35°C. as it is obvious that the temperature is dangerously high for carrot tissue. The same results are obtained as with potato and swede but in a more striking form. Thus, an increase in susceptibility to both fungi which approaches significance is obtained, even after 1 day's exposure to 30°C., and after 3 days of such exposure the rate of attack is more than doubled.

Effect of maturity of tubers on amount of attack

Inoculation experiments at different periods of growth and maturity

To observe the effect of age on the susceptibility of potato tubers to the attack of various rot-producing fungi, seven varieties of potato were used, namely, Epicure, Sharpe's Express, Arran Pilot, Great Scot, Arran Banner, Up-to-Date and King Edward. The first three are earlies, Great Scot a second early and the last three main crops. The various stages of growth and maturity at which the tubers were inoculated were as follows:

1*st. stage* i.e., when the tubers are young, undeveloped and on the average 1½"–2" in diameter; with early varieties such tubers were obtained in early July from plantings in mid April, in late varieties in the last week of July and Great Scot in mid July.

2*nd. stage* i.e., when the tubers are fairly large, but not fully developed; the tubers, in this case, were lifted 3 week after the first stage.

3*rd. stage* i.e., when the tubers are fully ripe, viz., mid to late August for earlies, mid to late September for the others.

4*th. stage* i.e., when the tubers are dormant—early to mid January.

5*th. stage* i.e., when the tubers begin sprouting—April to May.

All the above varieties were grown at the Imperial College Field Station, Slough in the summer of 1946. Thus, the same stock of material was used through the whole experiment and the possible error arising from the use of different stocks at different stages was eliminated.

The data of these experiments have been assembled in Table 6 and represent as usual the average amount of rotting produced in 10 inoculations after 6 days incubation at 20°C.

Table 8 shows that there is no significant difference in resistance between varieties tested. They behave alike so far as susceptibility to attack by the various fungi at different stages of growth and maturity is concerned. As regards the behaviour of the various fungi, the results may be summarised as follows :—

Botrytis cinerea, *Fusarium fructigenum* and *Rhizopus nigricans* which are not normally parasitic on potato tubers, actively produce rotting when the tubers are young and immature, i.e., in the 1st. and 2nd. stages and also when they are in the sprouting stage. The level of attack is comparatively low when the tubers are in the 3rd. stage and it is practically

negligible when they are dormant. Susceptibility decreases gradually from the 1st. to the 4th. stage, then suddenly jumps to a high figure when the tubers have sprouted.

The two fungi which are normal parasites of potato, namely *Pythium de baryanum* and *Phytophthora erythroseptica*, behave differently. Attack is much reduced on the younger tubers and tends to increase gradually until the dormant stage of the tuber is reached. The progressive increase is regular with *Pythium de baryanum*, less regular with *Phytophthora erythroseptica*. With both there is finally a pronounced increase in attack on the sprouted tubers.

Table 6. *Effect of maturity on susceptibility of different varieties of potato*

Variety	Stages of growth and maturity	Water Content %	*B. cinerea*	*F. fructigenum*	*R. nigricans*	*P. de baryanum*	*P. erythroseptica*
Epicure Potato	1st	84.2	0.309± 0.025	0.364± 0.01	0.318± 0.025	0.310± 0.027	0.73± 0.16
	2nd	84	0.374± 0.03	0.430± 0.03	0.359± 0.02	0.481± 0.02	1.915± 0.22
	3rd	83.6	0.287± 0.01	0.293± 0.023	0.230± 0.02	0.587± 0.02	1.533± 0.10
	4th	79.1	0.150± 0.02	0.142± 0.01	0.068± 0.02	0.720± 0.04	1.586± 0.12
	5th	77.5	0.624± 0.042	0.515± 0.03	0.36± 0.03	1.69± 0.13	4.64± 0.24
Sharpe's Express Potato	1st	80.2	0.424± 0.02	0.452± 0.02	0.331± 0.02	0.346± 0.02	0.929± 0.08
	2nd	79.6	0.501± 0.034	0.642± 0.02	0.526± 0.03	0.553± 0.02	1.92± 0.16
	3rd	79	0.278± 0.028	0.243± 0.014	0.25± 0.023	0.63± 0.04	1.622± 0.10
	4th	77	0.096± 0.01	0.108± 0.02	0.05± 0.01	0.773± 0.03	2.347± 0.25
	5th	74.2	0.787± 0.03	0.575± 0.04	0.470± 0.03	1.273± 0.08	4.829± 0.13
Arran Pilot Potato	1st	81	0.380± 0.01	0.418± 0.03	0.345± 0.05	0.40± 0.04	0.84± 0.05
	2nd	80.7	0.562± 0.025	0.731± 0.04	0.54± 0.02	0.565± 0.03	2.064± 0.26
	3rd	80.4	0.26± 0.02	0.272± 0.023	0.243± 0.025	0.607± 0.022	1.651± 0.18
	4th	78.6	0.125± 0.02	0.124± 0.013	0.04± 0.02	0.710± 0.03	2.363± 0.19
	5th	74.2	0.77± 0.03	0.536± 0.03	0.417± 0.012	1.03± 0.09	5.131± 0.28

Table 6. *contd.*

Variety	Stages of growth and maturity	Water Content %	*B. cinerea*	*F. fructi-genum*	*R. nigricans*	*P. de bary-anum*	*P. erythro-septica*
Great Scot Potato	1st	81.8	0.443± 0.03	0.48± 0.04	0.36± 0.035	0.427± 0.03	0.862± 0.05
	2nd	80.9	0.473± 0.03	0.487± 0.03	0.436± 0.04	0.05± 0.02	2.479± 0.087
	3rd	80.2	0.259± 0.026	0.224± 0.032	0.241± 0.04	0.60± 0.14	1.395± 0.13
	4th	77.2	0.102± 0.011	0.08± 0.01	0.062± 0.01	0.819± 0.093	1.883± 0.18
	5th	74	0.86± 0.04	0.60± 0.03	0.412± 0.024	1.450± 0.07	4.868± 0.35
Arran Banner Potato	1st	83.6	0.447± 0.03	0.484± 0.03	0.384± 0.02	0.388 ± 0.02	1.141± 0.16
	2nd	82.6	0.505± 0.03	0.538± 0.023	0.416± 0.034	0.576 ± 0.033	1.882± 0.16
	3rd	82	0.254± 0.024	0.286 ± 0.026	0.241± 0.018	0.603 ± 0.03	1.427 ± 0.12
	4th	77.1	0.121 ± 0.01	0.101 ± 0.015	0.06 ± 0.01	0.711± 0.11	2.016 ± 0.14
	5th	74.6	0.73± 0.05	0.530± 0.025	0.427± 0.02	1.251± 0.09	4.362± 0.25
Up-to-date Potato	1st	84.7	0.382± 0.016	0.401 ± 0.03	0.345± 0.024	0.408± 0.03	1.053± 0.15
	2nd	84	0.416 ± 0.032	0.495± 0.04	0.377 ± 0.02	0.601± 0.035	1.877± 0.15
	3rd	83	0.251± 0.024	0.255 ± 0.023	0.271 ± 0.016	0.623± 0.035	1.541± 0.17
	4th	79	0.125± 0.02	0.100± 0.013	0.07± 0.02	0.645± 0.036	1.893± 0.25
	5th	76.4	0.584± 0.03	0.513± 0.026	0.356 ± 0.028	1.166 ± 0.08	4.338± 0.24
King Edward Potato	1st	81	0.407 ± 0.025	0.44± 0.027	0.376± 0.014	0.409± 0.014	1.004± 0.074
	2nd	80.8	0.414 ± 0.209	0.593 ± 0.034	0.406± 0.025	0.58± 0.027	1.782± 0.17
	3rd	80	0.245± 0.023	0.278± 0.024	0.272 ± 0.02	0.585 ± 0.028	1.579± 0.05
	4th	77	0.044 ± 0.003	0.083± 0.015	0.026 ± 0.001	0.605± 0.013	1.909± 0.13
	5th	75.1	0.765± 0.042	0.591 ± 0.012	0.364± 0.02	2.37± 0.14	4.247± 0.15

Table 7. *Analysis of variance* of Table 6

Due to	Degrees of Freedom	Variance Ratio (F)	Probability
Variety	6	0.83	Not sig.
Fungus	4	466.8	$P<0.01$
Stages of Development	4	195.03	„ „
Variety × Fungus	24	0.34	Not sig.
Variety × Stages of Development	24	1.25	„ „
Fungus × Stages of Development	16	39.45	$P<0.01$
Error	96		

The water content of tubers gradually decreases as they pass from the 1st. to the 5th. stage. The steady fall in attack by three "non-parasites" as the tubers develop to the dormant stage may be due to the effect on these fungi on water content though this is probably not the only factor. The sudden rise as the tubers pass into the sprouting stage indicates the effect of a "maturity" factor which over rides the effect of a diminishing water content. The general behaviour of *Pythium de baryanum* accords with the effect of water content on attack by that fungus (Chona, 1932); in this case "maturity" and water content factors work together. The trend for *Phytophthora* attack is on the whole parallel to that of *Pythium*, but there are several ups and downs which would require further investigation.

Effect of dormant and post-dormant stages on susceptibility

It was shown earlier in this section that there is a considerable increase in susceptibility to rotting when the tubers pass from the dormant to the sprouting stage. It was interesting to see whether any such change takes place when dormancy is broken quite early by artificial means.

In January, 1947, dormant tubers were treated by the following methods in order to stimulate sprouting :—

(1) *Peeling method of Appleman* (1918).—The skin of the tubers was peeled off over almost the whole surface except for the region of the eyes. They were then stored in more or less dry saw dust in tin boxes at 20°C. (2) *Ethylene Chlorohydrin method of Denny* (1926).—Whole tubers were exposed to the vapour of ethylene chlorohydrin for 24 hours in air-tight glass jars (0.4 cc. of 100% solution per litre volume). After the treatment they were kept in moist saw dust in tin boxes at 20°C. (3) *Keeping in wet sawdust.*—Tubers slightly peeled at 2 or 3 places were stored in wet sawdust at 20°C. A set of controls was also kept in tin boxes at 20°C. Two different varieties were used in this connection, namely, King Edward and Majestic.

The treated tubers began to sprout vigorously within a fortnight, the sprouts reaching $\frac{1}{2}''$–$1\frac{1}{4}''$ in length. The peeling method gave the best results as regards both number and length of the sprouts. Treatment with ethylene chlorohydrin had the effect of producing a large number of short sprouts per tuber. The last method gave a few (1–2) sprouts per tuber. The controls remained unsprouted. Sprouts were not removed at the

time of inoculation The water contents of tubers after the various treatments were determined and are included in the Tables. The general conclusions to be drawn from Table 8 are as follows :

When the tubers are brought to the sprouting stage, they become more susceptible to attack than when they are dormant. There is no difference between the two varieties in this respect.

Tubers treated in the three different ways for breaking dormancy do not differ significantly from each other in susceptibility.

Table 8. *Effect of dormant and post-dormant stages on susceptibility of two varieties of potato.—Analysis of variance*

Due to	Degrees of Freedom	Variance Ratio (F)	Probability
Variety	1	1.45	Not Sig.
Treatment	3	40.03	$P<0.01$
Fungus	4	137.8	„ „
Variety × Treatment	3	0.70	Not Sig.
Variety × Fungus	4	0.001	„ „
Fungus × Treatment	12	3.28	$P=0.05$
Error	12	——	——

Physiological effects arising in certain conditions of host tissue

It is evident from the experiments described before, that under a variety of conditions there is a change in the susceptibility of the host tissue to the attack of a number of fungi, and it may be readily assumed that such change is due to some altered physiological condition of the cells leading to a higher degree of attack by various organisms. The present section records a series of experiments and observations which were designed to determine more precisely what these physiological conditions are.

Effect of temperature

Attention has been directed to the undermentioned features of the host cells, viz., (1) vitality, (2) water absorbing capacity, (3) changes in pH, (4) changes in cell-wall, and (5) changes in the diffusion of nutrients from the cells and the effect of these nutrients on germination, growth and enzyme secretion of certain fungi. These features were studied because they seemed likely to be responsible for the increase in susceptibility to rotting which results from pre-treatment of the tissue at some what high temperatures. The materials used in this connection were the potato varieties King Edward and Majestic, and swede.

(a) Changes in vitality of cells

The method of study was that of plasmolysis by 10% KNO_3, but the details were different for the two tissues, potato and swede, which were

used. The cells of potato are so full of starch inclusions that direct observation of plasmolysis under the microsope could not be carried out. Swede tissue is, however, amenable to such examination.

Shallow cylinders of potato when placed in the hypertonic solution became flaccid and pliable. On being now placed in water they became turgid and stiff, thus indicating substantial recovery from plasmolysis. By applying this test to cylinders cut from potatoes which had been stored at 35°C. for 15 days (the severest treatment adopted) it was found that the cylinders which were limp and desiccated after the treatment, became firm when placed in water. Thus, though the individual cells were not under observation, the tissue as a whole reacted in the normal living manner.

The same general conclusion can be drawn from the fact that potato tubers, even after 15 days at 35°C., later behave in a completely normal manner, e.g., they sprout as usual and on being cut across they show no abnormal features. In other words, as far as can be seen, the heat treatment adopted does not damage cells of potato tissue.

With swede, which is suitable for microscopic examination, the procedure was as follows. Two thin free-hand sections were taken from each of the three different regions of swede, namely, apex, base and centre. After being kept in 10% KNO_3 solution for some time, they were taken out and examined under the microscope. In each section, at three different places chosen at random, the number of plasmolysed cells in the field of the microscope was counted. In each particular set of observations, sections from five different swedes were taken. Examinations were made of cells of swede tissue stored at 35°C. for 1, 3, 5 and 7 days together with those of the controls stored at 15°C. for similar times. The data are set out in Table 9. The Table shows that no change in the proportion of non-plasmolysable cells had resulted from the heat traeatment until after about 5 days. At this stage, there is a slight increase which is non-significant but after a further exposure of two days the change is considerable and significant. It may be mentioned that cells from the centre of the roots are the most sensitive in this respect. Swede tissue is thus more liable to damage by heat treatment than potato tissue, as was pointed out before in connexion with studies on susceptibility to fungal invasion.

Table 9. *Effect of pre-storage on vitality of swede cells stored at* 35°C. *and* 15°C.

Pre-storage Temperature	Days				
	1	2	3	5	7
35°C.	3.15*	3.13	3.88	4.13	16.07
15°C.	3.08	3.47	3.42	3.12	3.35

* percentage of non-plasmolysed cell.

(b) CHANGES IN WATER-ABSORBING CAPACITY OF CELLS

From a cylinder of potato (1.8 cm. in diameter) ten 0.5 cm. pieces were cut, rapidly washed, dried and weighed. These were placed in distilled water for 24 hours after which they were again dried and weighed. The differences in weight expressed in terms of percentage of the first weight has been taken as the water-absorbing capacity of the cells. The results of such an experiment with three varieties of potato are given in Table 10, the figures in the Table being averages of six sets of determinations.

Table 10. *Effect of pre-storage temperature on water-absorbing capacity*

Variety	Storage at 15°C. for		Storage at 35°C. for	
	3 days	7 days	3 days	7 days
King Edward	14.10±1.35	14.4 ±1.48	15.2 ±1.56	23.2 ±1.2
Majestic	14.7 ±0.58	14.7 ±0.5	16.1 ±0.64	21.1 ±.168

A significant increase in water-absorbing capacity is thus produced after 7 days' exposure to 35°C., all the varieties behaving similarly. This effect is no doubt due largely, if not entirely, to loss of water during the heat treatment and not to any material change in the contents of the cells of the treated tissue.

(c) CHANGES IN pH OF CELL-SAP

The H-ion concentration of the cell-sap of swede and potato stored at 15°C. and 35°C. for varying periods was determined colorimetrically. With both tissues, no change in pH of the cell sap due to storage at higher temperature was observed. The average pH of potato was 5.4–5.6 and of swede 4.2–4.4. Thus any possible role of pH in the changes in susceptibility can be ruled out.

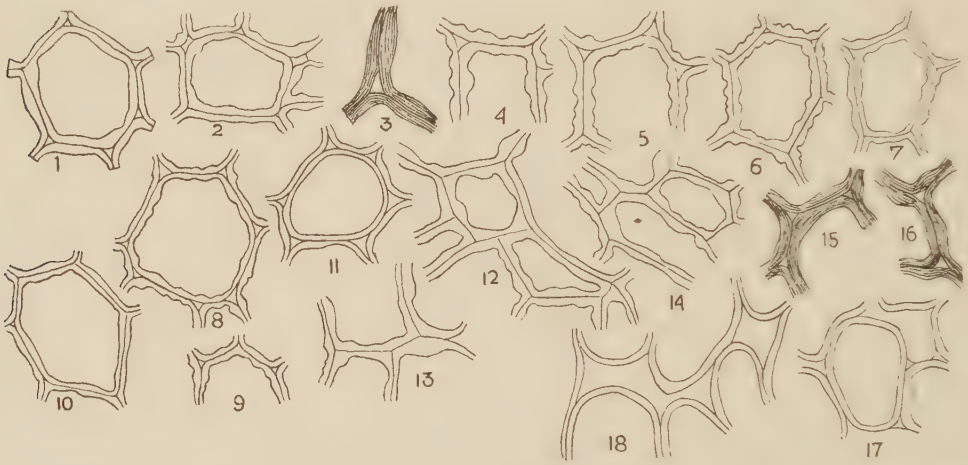
(d) CHANGES IN THE CELL-WALL

Changes in the cell-wall were studied in relation to staining reactions and to susceptibility to enzyme action.

(i) *Changes in the staining reaction of cell-walls.* Free-hand sections were cut from fresh material and stained with aqueous solution of Ruthenium Red. Pectic substances appear red after staining.

Potato. Observations were made on tissues of the varieties King Edward and Majestic which had been stored at 15°C. and 35°C. for 1–15 days. Tissue which was not exposed to the higher temperature shows, after staining, a distinct red line in the position of the middle lamella. Throughout the wall proper there is an alternation of red-stained and unstained bands. This is shown in black in fig. 3. of Text-fig.2. The walls at this stage are comparatively thick (Text-figs. 2. figs. 1–3). With tubers which are stored at 35°C., no recognisable change occurs until after about 7–10 days. Thereafter the walls become thinner in an irregular manner so that they take on a some what wavy appearance (Text-fig. 2., figs. 4-8). After 15 days storage 35°C., the walls are uniformly thin (Text fig. 2, figs. 9-11) but the distribution of red-staining material is not different from what it was at the beginning.

Swede. The cell-walls of unheated tissue stains generally and deeply with Ruthenium Red so that there is little constrast between the middle lamella and the rest of the wall. The general appearance of such a cell is shown in Text-fig. 2, figs. 12-14. When the material is stored at 35°C. changes are recognisable much earlier than with potato. Thus after 3 days, the wall ceases to stain uniformly but shows a streaked appearance (Text-fig. 2, figs. 15-16). After 7 days' treatment at 35°C., the walls are distinctly thinner and stain uniformly, but comparatively lightly with Ruthenium Red. There is also a considerable tendency of cells to break away from each other, so that the inter-cellular system is increased (Text-fig. 2, figs. 17-18). These effects are no doubt symptomatic of the breakdown which occurs after such treatment.



Text-fig. 2. 1-11, Changes in cell-wall of potato (King Edward) due to storage at 35°C : 1-3. normal, stored at 15°C. 4-8 stored at 35°C for 7-10 days. 9-11 stored at 35°C for 15 days 12-18. Changes in cell-wall of swede 12-14 normal stored at 15°C. 15-16, stored at 35°C for 3 days. 17-18, stored at 35°C for 7 days.

(ii) *Susceptibility to enzyme action.* This was tested in two ways : (1) by maceration test in which the time taken by a standard enzyme preparation to bring about loss of coherence in tissue discs of standard thickness was determined. The susceptibility is to be taken as inversely proportional to the time required for maceration. The loss of coherence was determined by taking the sections from time to time out of the solution and pulling gently with forceps. Three sections of standard thickness (1.8 cm. in diameter and 0.5 mm. in thickness) were usually placed in 3 cc. of the enzyme solution and the average time noted. In these tests, three sections from each of the ten pieces of tissues were used.

(2) by determining the amount of tissue rotted when a small quantity (0.4 cc.) of standard enzyme solution was placed in a cavity (prepared as in inoculations with spores,) and incubated for 24 hours at 20°C.

Experiments were carried out by both methods on tubers of King Edward and Majestic potatoes and by the first method on swede. For this work two different enzymic (pectinase) preparations were used, viz.,

the endoenzyme of *Botrytis cinerea* and the exoenzyme of *Pythium de baryanum*. The preparations were made in the manner already described.

With the endoenzyme of *B. cinerea*, it is possible to prepare a large quantity of powdered material so that the same stock can be used throughout the whole experiment. Thus, the possible error arising from differences in strength of enzyme preparations can largely be eliminated. With *Pythium de baryanum* this is not possible. Therefore, to have a maximum degree of uniformity, the enzyme was always extracted from inoculated potato cylinders after 15 days incubation at 20°C.

As usual the vegetables were stored at 15°C. and 35°C. and the period of storage was 1, 2, 3, 5, 7, 10 and 15 days for potato and 1, 2, 3, 5 and 7 days for swede. The results of the various observations are summarised in Tables 11–14.

Table 11. *Time (in minutes) for enzyme of* B. cinerea *to macerate discs of potato at* 35°C. *and* 15°C.

Variety	Storage Temp.	Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C	30± 0.42	26.7± 0.29	25.8± 0.53	24.2± 1.24	21.2± 0.42	20.8± 0.42	19.2± 0.68
	15°C	30.9± 0.55	30.2± 0.22	31.6± 1.73	31.4± 0.48	31.9± 0.52	31.4± 0.48	30.7± 0.64
Majestic	35°C.	33.0± 0.42	31.2± 0.44	25.8± 0.89	24.8± 0.43	23.1± 0.89	21.3± 0.91	17.5± 1.01
	15°C.	33.5± 0.55	33.7± 0.54	32.6± 0.82	33.0± 0.82	33.5± 0.47	32.9± 0.69	33.8± 0.51

Table 12. *Time for (in hours) enzyme of* P. de baryanum *to macerate discs of potato pre-stored at* 35°C. *and* 15°C.

Variety	Storage Temp.	Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	6	6	5	5	4.5	4.5	3.5
	15°C.	6	5.5–6	6.5	6	6	6	6
Majestic	35°C.	5.5–6	5.5–6	3	3	2.5–2.8	3	2.5
	15°C.	5.5–6	5.5–6	4.5	4.5	4	5	4

Table 13. *Time for enzyme of* B. cinerea *and* P. de baryanum *to macerate discs of swede pre-stored at* 35°C. *and* 15°C.

Storage Temp.	Enzyme of	Period of Storage (Days) 1	2	3	5	7
35°C.	*B. cinerea*	57± 1.3 min.	44± 1.9 min.	40± 2.0 min.	30± 1.4 min.	26± 0.8 min.
	P. de baryanum	8 hrs.	8 hrs.	7 hrs.	6.5 hrs.	6 hrs.
15°C.	*B. cinerea*	58± 2.1 min.	62± 1.7 min.	60± 0.7 min.	61± 1.1 min.	66± 3.1 min.
	P. de baryanum	8 hrs.	8 hrs.	8 hrs.	8 hrs.	8 hrs.

Table 14. *Average amount (grms.) of rotted tissue produced in 24 hours by a definite quantity of enzyme of* B. cinerea *and* P. de baryanum *on tubers pre-stored at* 35°C. *and* 15°C.

Variety	Storage Temp.	Enzyme of	Period of Storage (Days)						
			1	2	3	5	7	10	15
King Edward	35°C.	*B. cinerea*	0.315 ± 0.018	0.336 ± 0.012	0.45 ± 0.02	0.53 ± 0.025	0.57 ± 0.03	0.632 ± 0.015	0.77 ± 0.034
		P. de baryanum	0.189 ± 0.011	0.201 ± 0.007	0.251 ± 0.01	0.307 ± 0.01	0.341 ± 0.013	0.353 ± 0.008	0.44 ± 0.015
	15°C.	*B. cinerea*	0.296 ± 0.023	0.29 ± 0.017	0.288 ± 0.009	0.30 ± 0.02	0.298 ± 0.015	0.283 ± 0.008	0.286 ± 0.013
		P. de baryanum	0.177 ± 0.006	0.181 ± 0.013	0.179 ± 0.011	0.182 ± 0.012	0.189 ± 0.011	0.195 ± 0.01	0.193 ± 0.011
Majestic	35°C.	*B. cinerea*	0.294 ± 0.012	0.341 ± 0.016	0.528 ± 0.017	0.568 ± 0.026	0.744 ± 0.05	0.781 ± 0.048	0.820 ± 0.023
		P. de baryanum	0.180 ± 0.008	0.206 ± 0.007	0.291 ± 0.012	0.301 ± 0.032	0.351 ± 0.012	0.389 ± 0.027	0.499 ± 0.03
	15°C.	*B. cinerea*	0.298 ± 0.009	0.304 ± 0.013	0.281 ± 0.02	0.298 ± 0.028	0.309 ± 0.021	0.294 ± 0.016	0.294 ± 0.014
		P. de baryanum	0.176 ± 0.007	0.177 ± 0.012	0.181 ± 0.016	0.180 ± 0.012	0.184 ± 0.010	0.188 ± 0.011	0.178 ± 0.016

The experiments of Tables 11-14 agree in showing that storage of the tissues used at a temperature of 35°C. increase susceptibility to attack by the enzyme of *Botrytis cinerea* and *Pythium de baryanum*. To this effect must be ascribed a part at least of the enhanced susceptibility shown by these tissues after heat pre-treatment to attack by the organisms themselves. It is clear that the heat treatment has brought about a change in the nature of the pectic cell-wall constituents, rendering them more readily hydrolysable, and thus confirming the changes which were noted above by the staining technique. That pectinase enzymes play an important role in the hydrolysis of pectin during ripening and senescence has been observed by a number of workers, e.g., Thatcher (1915) and Atkins (1916) on the ripening of fruits, and Carré (1925) on the ripening of apple.

(e) CHANGES IN EXOSMOSIS OF NUTRIENS AND THEIR EFFECT ON GERMINATION, GROWTH AND ENZYME SECRETION OF CERTAIN FUNGI.

Increased attack by fungi is brought about when nutrient material is supplied to the spores or mycelium as was shown, for example, by Brown (1922) in experiments on the exosmosis of substances from plant structures (leaves, petals) and on the effects of those upon germination of spores of *Botrytis cinerea*. The obvious suggestion, therefore, was to test whether heat pre-treatment which has been shown to increase susceptibility has an effect on exosmosis from the cells so treated. Accordingly an investigation was taken up along the following lines : (i) estimation of total sugar, salts and total nitrogen in diffusates from host cells, (ii) influence of diffusates on germination of spores of *Botrytis cinerea*, and (iii) influence of diffusates on mycelial growth and enzyme (pectinase) secretion of *Botrytis cinerea* and *Pythium de baryanum*.

(i) *Estimation of total sugar, salts and total nitrogen in diffusates from host cells.* Sixteen cylinders (1.8 cm. in diameter and 1 cm. in height) were cut aseptically from tubers previously stored under different conditions. After being washed thoroughly with sterile distilled water to remove cell contents from the cut surface, they were transferred to 500 cc. conical flasks each containing 30 cc. of sterile distilled water. The flasks were kept at 20°C. for 24 hours after which the liquid was taken out and made up to the original volume of 30 cc. Then the amounts of total sugar, salts and nitrogen present in the liquid were determined by the following methods.

Sugar. 5 cc. of the liquid was used for this purpose. Total sugar was determined after inversion of any sucrose present, by Plank's method (1936) using the reagent of Harding and Down (1933). To correct for any reducing substances other than sugar which might be present, 5 cc. of the solution were kept at 37°C. for 24 hours with 5 cc. of yeast suspension. This removes all fermentable sugars and thus an estimate can be made by subtraction for reducing substances other than these. In actual fact, the only reducing substances present were sugars.

Salts. For this purpose, 15 cc. of the liquid was diluted with 15 cc. of distilled water and the conductivity of the solution determined by use of the "Conductivity Bridge Method" of the Cambridge Scientific Instru-

ment Company. The increase in conductivity is proportional to the increase in amount of salts.

Total Nitrogen. This was determined by the micro-Kjeldahl method with 2 cc. of the solution.

These determinations were made with Majestic potato tubers only. Two ranges of temperatures, namely 35°C. and 15°C. were used and the periods of storage were 1, 3, 5, 7, 10 and 15 days. The results in all cases represent an average of three-fold replications.

The data of the sugar estimations are given in Table 15.

Table 15. *Amount of total sugar (mg.) present in* 100 cc. *of diffusates from cells of potato* (var. Majestic)

Pre-storage Period (Days)	1	3	5	7	10	15
35°C.	27.03± 1.14	43.7± 3.8	59.8± 2.08	70.0± 3.3	89.0± 7.6	162.5± 1.5
15°C.	24.2± 0.5	25.3± 1.0	26.4± 1.1	24.2± 0.5	26.3± 1.1	26.0± 0.7

Even after 1 day storage at 35°C., exosmosis of sugar is increased, and after 3 days, the increase is highly significant. With longer exposure the rate of exosmosis becomes progressively faster.

Table 16 gives the data for total salts which have diffused out. The figures in the Table represent relative electrical conductivities ($\times 10^3$) of the diffusates.

Table 16. *Total amount of salts present in the solution expressed in terms of conductivity*

Pre-storage Period (Days)	1	3	5	7	10	15
35°C.	0.27± 0.004	0.34± 0.028	0.46± 0.032	0.52± 0.037	0.52± 0.03	1.02± 0.09
15°C.	0.25± 0.006	0.26± 0.011	0.24± 0.011	0.24± 0.011	0.25± 0.01	0.23± 0.011

The results of Table 16 follow the same lines as those for total sugar, viz., a steady increase with exposure to heat treatment. A statistically significant increase is reached by the 5th day of pre-storage. No nitrogenous substances were found in any of the solutions examined.

The leaching out of salts and sugar is no doubt primarily due to increased permeability of the plasma membrane resulting from pre-treatment at the higher temperature. Regarding sugar, there is the further possibility that under the influence of high temperature there may have been rapid hydrolysis of starch resulting in accumulation of sugar.

(ii) *Influence of diffusates on germination of spores of* Botrytis cinerea. In order to obtain diffusates in a more concentrated form than by the method

described before, the following procedure was adopted. The cylinders of tissue (1.8 cm.×3 cm. deep) were first washed as before, then each was fitted at the upper end with a sterile rubber ring arranged so as to make a water-holding chamber. Into the latter was put 0.5 cc. of sterile distilled water. The cylinders were kept in moist dishes at 20°C. After 24 hours, a drop was taken from each on a clean slide and a drop of spore-suspension added to it. The latter was so prepared that when mixed with the drop of diffusate there were about 30–40 spores in the field of the microscope. The spores were taken from cultures 10–14 days old to eliminate irregularities due to ageing. Distilled water from the same stock and treated similarly was used as controls.

The slides were incubated at 20°C. for 15–16 hours after which the average germ tube length was determined. This was based on measurements of 50 spores chosen at random from each preparation. With diffusates from potato and swede, the number of slides recorded was 8 and 5 respectively for each treatment. Tables 17 to 19 give the results of these experiments.

Table 17. *Effect of diffusates from* King Edward potato *on germ-tube length* (μ) *of* B. cinerea.

Pre-storage Period→ (Days)	1	2	3	5	7	10	15
35°C.	56.0± 0.9	63.9± 2.2	80.5± 5.1	123.3± 6.0	152.0± 4.5	175.5± 6.7	201.5± 5.0
15°C.	53.6± 1.8	49.0± 1.4	47.7± .12	50.1± 3.4	49.0± 2.2	50.0± 3.7	45.9± 2.0
Control in distilled water.	34.5± 3.6	32.0± 3.9	32.7± 1.7	30.5± 2.7	30.0± 1.0	30.1± 2.9	31.4± 2.8

Table 18. *Effect of diffusates from* Majestic potato *on germ-tube lengths* (μ) *of* B. cinerea

Pre-storage Period→ (Days)	1	2	3	5	7	10	15
35°C.	62.0± 2.1	79.0± 3.2	104.5± 3.9	118.3± 4.4	160.0± 6.0	190.4± 6.7	232.0± 7.7
15°C.	60.0± 4.0	58.7± 1.7	56.0± 2.0	60.0± 1.9	60.5± 3.4	64.8± 1.7	57.8± 2.5
Control in distilled water.	41.2± 2.0	39.5± 2.0	37.1± 2.1	40.7± 1.8	38.1± 1.3	36.9± 1.3	36.9± 1.8

Table 19. *Effect of diffusates from swede on germ-tube length* (μ) *of* B. cinerea

Pre-storage period→ (Days)	1	2	3	5	7
35°C.	124.2± 3.6	140.0± 6.5	212.4± 8.7	276.0± 10.5	368.0± 18.0
15°C.	103.6± 4.5	107.0± 2.5	105.1± 4.7	108.4± 6.4	102.1± 4.4
Control in distilled water.	39.3± 3.1	40.1± 5.1	45.5± 7.1	49.3± 3.9	51.9± 3.2

It is obvious from Tables 17–19 that water kept in contact with tissue previously stored at 35°C. has a greater stimulatory effect on the germination of spores than has water in contact with the controls. This effect is indicated after 2 days' pre-treatment of potatoes and with swede after 1 day's treatment. This enhancement in germination is progressive with lengthened period of storage at 35°C. Compared with distilled water, the liquid which was lying in contact with cells of potato and swede previously stored at 15°C. gives increased germination, but in this case, the period of storage has no effect.

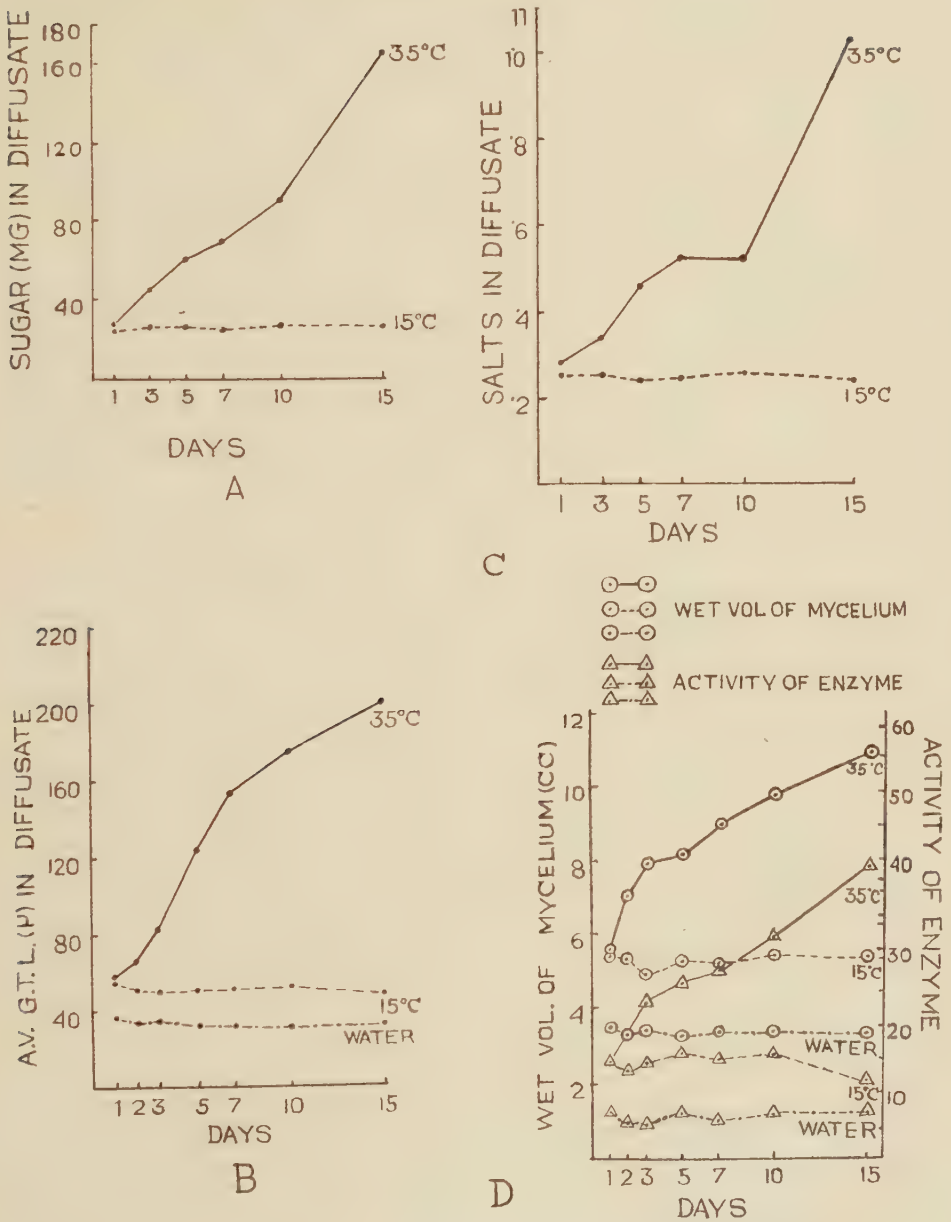
One may confidently assume that these stimulative effects on germination arise from the increased exosmosis of sugar and salts from the pre-treated tissues. The latter result has been already demonstrated by chemical and physical methods.

(iii) *Influence of diffusates on mycelial growth and enzyme (pectinase) secretion of* Botrytis cinerea *and* Pythium de baryanum. Tissue cylinders were out from control and treated potatoes and swedes and kept in 30 cc. of distilled water in 500 cc. conical flasks for 24 hours at 20°C. The liquid was then taken out and made up to the original volume.

With *Botrytis cinerea*, Brown's plate method (1915) was used for the study on growth and enzyme production. The 30 cc. of diffusate after being autoclaved was mixed with a definite quantity of spores (0.1 cc. of wet volume of spores to 10 cc. of the liquid), then pipetted on to circular glass plates (8″ in diameter) at the rate of 10 cc. per plate. After 48 hours, the liquid was taken off and the mycelium collected. As the amount of growth was small, dry weight determinations were inconvenient so that recourse was had to wet volume measurement by displacement of water. The activity of the exoenzyme was determined by the usual disc method. These experiments were run with control and treated tubers, the latter pre-stored at 35°C., and also distilled water. The last served as an additional control.

With *Pythium de baryanum*, the liquid (30 cc.) was divided equally between three 100 cc. flasks. After sterilisation the flasks were inoculated from a 4–6 day's old culture of *Pythium de baryanum* and incubated for 7 days at 20°C. The mycelial mat was then collected and its dry weight determined. The liquid was tested for the enzyme pectinase in the usual way. In this case no control with distilled water was attempted.

The results are given in Tables 20 and 21, the figures being the averages of three replicates. The activity of the enzyme preparations of *B. cinerea* has been expressed as the inverse of the time required for maceration, the activity corresponding to a maceration time of 30 minutes being taken as 100. In Table 21, which relates to *Pythium de baryanum*, the records of activity are expressed in hours.



Text-fig. 3. A. Amount of total sugar (mg.) present in 100 c.c. of diffusates from cells of Majestic potato pre-stored at 35°C and 15°C for 1–15 days. B. Average germ-tube length (μ) of *B. cinerea* in diffusates from cells of King Edward potato pre-stored at 35°C and 15°C for 1-15 days and in water. C. Amount of salts (electrical conductivity $\times 10^3$) in diffusates from cells of Majestic potato pre-stored at 35°C and 15°C for 1–15 days. D. Wet volume of mycelium (c.c.) and enzymic activity of *B. cinerea* (maceration time in hours) when grown in diffusates from cells of King Edward potato pre-stored at 35°C and 15°C for 1–15 days and in water.

Table 20. *Growth and enzymic activity of* B. cinerea *in diffusates* (*Wet volume and mycelium in c.c.*)

Potato variety	Pre-storage Temp.		Preiod of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	Mycel.	0.56	0.71	0.80	0.83	0.91	0.98	1.20
		Enzyme	13	18	21	24	25	30	40
	15°C.	Mycel.	0.55	0.54	0.50	0.54	0.52	0.55	0.56
		Enzyme	13	12	13	14	13	14	11
	Water control	Mycel.	0.35	0.34	0.35	0.33	0.34	0.34	0.35
		Enzyme	6	5	5	6	5	6	6
Majestic	35°C.	Mycel.	0.52	0.60	0.75	0.88	0.91	1.05	1.30
		Enzyme	12	15	19	25	25	32	43
	15°C.	Mycel.	0.53	0.50	0.51	0.50	0.56	0.56	0.60
		Enzyme	13	12	12	14	13	13	16
	Water control	Mycel.	0.36	0.37	0.40	0.38	0.33	0.40	0.42
		Enzyme	7	6	6	6	5	6	7
Swede	35°C.	Mycel.	1.52	2.3	3.0	3.45	3.89	—	—
		Enzyme	10	10	11	15	21	—	—
	15°C.	Mycel.	0.83	0.85	0.90	0.90	0.85	—	—
		Enzyme	10	10	11	10	10	—	—
	Water control	Mycel.	0.33	0.35	0.34	0.35	0.34	—	—
		Enzyme	5	5	5	6	5	—	—

Table 21. *Growth and enzyme activity of* P. de baryanum *in diffusates* (*Dry weight in mg.*)

Potato variety	Pre-storage Temp.		Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	Mycel.	5.3	8.4	9.0	10.3	11.0	12.7	15.6
		Enzyme	7.5	8	7	6.5	6.5	6.0	6.0
	15°C.	Mycel.	5.1	5.5	5.3	5.4	5.7	6.0	6.0
		Enzyme	7.5	8	7.5	7.5	8	8	7.5
Majestic	35°C.	Mycel.	5.3	6.0	7.4	8.3	9.6	11.3	13.7
		Enzyme	8	8.5	7	6.5	6.5	5	5
	15°C.	Mycel.	5.0	4.7	5.0	5.3	5.0	5.4	5.5
		Enzyme	8	8.5	8	8	8.5	7.5	8
Swede	35°C.	Mycel.	9	13	14.5	16.6	18	—	—
		Enzyme		20–24 hours in all cases					
	15°C.	Mycel.	7.5	8	7.6	8.2	9	—	—
		Enzyme		20–24 hours in all cases					

The general summary of Tables 20 and 21 shows that with increasing time of pre-storage at 35°C., the diffusates obtained give increased mycelial growth of *Botrytis cinerea* and *Pythium de baryanum*. Almost invariably there is a concomitant increase in the amount of exo-enzyme secreted,

the only apparent exception being in the case of *Pythium de baryanum* on swede diffusate where any variation of enzymic activity could not be established on account of the low activity shown.

Effect of age

In order to allow of comparisons being made at the same time between tubers of different ages, a batch (vars. King Edward and Majestic) was placed in cold store and thus kept in the dormant condition from early Spring 1947 until June. Meanwhile plantings were made in the field from the same stocks. In June, some of the cold-stored tubers were taken out peeled and thus stimulated to sprout. By the beginning of July, one had on hand tubers at three stages of advancement, viz., young tubers of the 1947 crop, and dormant and sprouted tubers held over from 1946. Observations were made on these with regard to cell wall and permeability changes.

(a) CHANGES IN THE CELL-WALL

Microscopic observations after staining sections with Ruthenium red did not indicate any major differences in the pectic constituents of the cell-wall in tubers of different ages. The only difference observed was a somewhat deeper staining of the walls of the young tubers.

Susceptibility to enzyme action was examined by the two methods already described, the endo-enzyme of *Botrytis cinerea* and exo-enzyme of *Pythium de baryanum* being used.

There was no difference between the tubers of different ages so far as susceptibility of cell-well to enzyme action is concerned. Thus it is indecated that changes in susceptibility of the tubers to attack at different stages of development and maturity are not due to any differences in the condition of the cell-wall.

(b) CHANGES IN THE EXOSMOSIS OF NUTRIENTS AND THEIR EFFECT ON GERMINATION, GROWTH AND ENZYME SECRETION OF CERTAIN FUNGI

The studies in this connection were carried out in the manner already described. Table 22 gives the data for exosomosis of sugar, salts and nitrogen from tubers of different ages.

The results of Table 23 indicate that much greater exosmosis of solutes takes place from young and sprouting than from dormant tubers. Further investigation would be required to determin whether this effect is due to greater permeability of the protoplasts or to a greater proportion of the cell metabolites being in a mobile condition in the form case.

The effect of the diffusates from young, dormant and sprouting tubers on spore germination is shown in Table 22 and that on mycelial growth and enzyme production in Table 24.

Table 22. *Estimation of sugar, salts* and nitrogen in diffusates (mg. per 100 c.c.) from tubers of different ages*

Variety		Young	Dromant	Sprouting
King Edward	Total Sugar	224.5± 4.0	18.3± 1.1	93.3± 4.0
	Total Salt *	3.27± 0.15	0.29± 0.02	1.08± 0.11
	Total nitrogen	Trace	Not detected	Trace
Majestic	Total sugar	185.7± 7.7	14.6± 1.1	79.4± 2.6
	Total salt *	3.05± 0.03	0.32± 0.03	0.80± 0.024
	Total nitrogen	Trace	Not detected	Trace

* conductivity $\times 10^3$

Table 23. *Effect of diffusates on germ-tube lenyth (μ) of* Botrytis cinerea

	Young	Dormant	Sprouting	Control in water
King Edward	weft	54.0±2.3	weft	28.5±1.2
Majestic	weft	62.7±3.2	weft	30.0±1.5

The results of Tables 23 and 24, confirm those of Table 22 in showing that diffusates from young and sprouted tubers strongly surpass those from dormant tubers in their effect on fungal germination and growth. A parallel effect is seen on enzyme production, clearly in the case of *Botrytis cinerea*, less clearly in the case of *Pythium de baryanum.*

Table 24. *Effect of diffusates on mycelial growth and enzyme production*

Variety			Young	Dormant	Sprouting
King Edward	*B. cinerea*	Wet vol. of mycel. (cc.)	2.1	0.68	1.8
	P. de baryanum	Dry wt. of mycel. (mg.)	34	10	25
		Activity of exo-enyme (hrs.)	6.5	6.5	6.5
Majestic	*B. cinerea*	Wet vol. of mycel. (cc.)	2.5	0.65	2.1
		Activity of exo-enzyme	25	11	20
	P. de baryanum	Dry wt. of mycel. (mg.)	40	8	31
		Activity of exo-enzyme (hrs.)	7	6.5	3.5

Comparison between *Botrytis cinerea* and *Pythium de baryanum* in parasitic behaviour

It has been shown above that *Botrytis cinerea* does not attack dormant potato tubers to any extent, whereas *Pythium de baryanum* produces an appreciable amount of rotting. Conversely, *Pythium de baryanum* does not attack swede, or carrot, but *Botrytis cinerea* behaves as a normal parasite on these vegetables. The question therefore arises whether these differential effects are due to the presence in these various tissues of substances antagonistic to the one but not to the other of these two fungi.

Effect of plant juices on germination, growth and enzyme secretion

That antagonistic substances do exist in plant tissues has been claimed by a number of workers. Thus, Schmidt (1933) has suggested that the basis of resistance of some species of *Solanum* to *Cladosporium fulvum* is the presence of an antagonistic principle probably solanin, in the sap. Reynolds (1931) states that flax strains resistant to *Fusarium lini* contain a higher percentage of HCN producing glucoside than do suceptibile strains. Rochlin (1934) in studying the non-susceptibility of some Cruciferae to *Plasmodiophora brassicae* has observed a direct relationship between the degree of resistance exhibited by a given species and the amount of glucosides which on fermentation produce highly pungent mustard oil. Link and Walker (1933) found that sap of coloured resistant onions is more toxic to the fungus *Colletrichum circinans* responsible for the "smudge" disease of onions. Storey (1941) working with potato and radish strains of *Rhizoctonia solani* showed that resistance of Cruciferous hosts to the isolate from potato is due to the presence of some substance in the cell which the appropriate (cruciferous) strains can tolerate. Berridge (1929) states that the fresh juice of potato possesses strong agglutinating action on certain bacteria. This action is due to the presence of some substances which can be precipitated out of the potato juice by 95% alcohol at the natural pH of the sap. The precipitate solution agglutinates and plasmolyses the non-pathogenic bacteria, but pathogenic ones remain unaffected.

Raw juice of potato, swede, carrot and turnip was prepared according to the method of Menon (1934). As it was undesirable to sterilise such preparations by autoclaving or steaming, which would throw down proteins and other colloids, and as classification by means of a Seitz filter proved to be too slow or practical purposes the following method was adopted.

The crude juice was pressed from the minced tissue, collected in a sterile flask, and heated to 60–65°C. or 10 minutes. It was then held at 0°C. or 24 hours and heated again as before. This process was repeated a third time. By this means a practicable degree of sterilisation was obtained without formation of precipitates or geling of starch.

Table 25 gives the germ-tube length of *Botrytis cinerea* in juices so prepared, the figures being the average of 5 replicates.

Table 25. *Germ-tube length* (μ) *of* Botrytis cinerea *in various juices and water*

Period of Germination	Potato	Carrot	Swede	Turnip	Water
16 hrs.	200.0± 8.8	198.0± 8.7	202.0± 6.1	213.5± 6.8	58.5± 3.7
40 hrs.	weft	weft	weft	weft	—

From these results, it is clear that potato juice has no depressing effect on the germination of spores of *B. cinerea*.

The amount of mycelial growth of *B. cinerea* and *P. de baryanum* in the various juices was determined by growing each fungus in 10 cc. of the liquid in 100 cc. flasks. The experiment was run in triplicate at 20°C. for 7 days. The data obtained are given in Table 26.

Table 26. *Mycelial growth* (*dry weight in gms.*) *of* B. cinerea *and* P. de baryanum *in various juices*

	Potato	Carrot	Swede	Turnip
Initial pH	5.4	5.2	4.4	4.4
B. cinerea	0.45± 0.03	0.56± 0.07	0.60± 0.04	0.52± 0.05
P. de baryanum	0.35± 0.02	0.15± 0.03	No growth	No growth

Thus *Botrytis cinerea* can grow well on all the plant juices. On the other hand, *Pythium de baryanum* grows well on potato and to a moderate extent on carrot, but not at all on swede or turnip. The absence of growth in the last two cases may be due to the low pH, because *Pythium de baryanum* is not favoured by acidity of the medium.

The ability of the fungi to secrete enzyme and the strength of the enzyme if secreted, were studied in the following ways :—

(1) With *Botrytis cinerea*, Brown's plate technique (1915) was adopted, After 48 hours' growth, the activity of the endo- as well as the exo-enzyme was determined in the usual way.

(2) With *Pythium de baryanum*, after 7 days' growth at 20°C., the liquid was tested for exo-enzyme.

Table 27 gives the result of the experiment. The strength of the enzyme has been expressed in terms of time taken to macerate the discs of potato. A 3-fold replication was used.

The outstanding result from Table 27 is that *Botrytis* produces a much weaker exo-enzyme on potato than on carrot and that the exo-enzyme of *Pythium* behaves in a converse manner.

Table 27. *Activity of enzyme secreted by* B. cinerea *and* P. de baryanum *in various plants juices*

	Potato	Carrot	Swede	Turnip
Initial pH	5.4	5.2	4.4	4.4
B. cinerea (exo.)	12 hrs.	1.5 hrs.	48 mins.	53 mins.
B. cinerea (endo.)	1.5 hrs.	45 mins.	35 mins.	35 mins.
P. de baryanum (exo)	3.5 hrs.	18–20 hrs.	No enzyme as there was no growth.	As for Swede

Effect of plant juices on enzyme activity

The results just described may be due in part to different rates of excretion of enzyme according to the medium used, but they are also to be explained (in part at least) by the fact that different plant juices have unequal retarding effects on the macerating action of the enzyme. This is shown by the results of Table 28. An enzyme solution was set up and a series of dilutions was made: (1) with water, (2) with various juices, and the activities of these dilutions compared.

Table 28. *Effect of plant juices on the activity of enzyme of* B. cinerea *and* P. de baryanum

Enzyme	Conc.	Diluting Liquid				
		Water	Potato	Carrot	Swede	Turnip
B. cinerea (endo.)	100%	35 mins.	—	—	—	—
	75%	40 „	130 mins.	70 mins.	65 mins.	65 mins.
	50%	55 „	11 hrs.	130 mins.	120 mins.	130 mins.
	25%	80 „	24 hrs.	300 mins.	280 mins.	320 mins.
P. de baryanum (exo.)	100%	210 mins.	—	—	—	—
	75%	260 „	300 mins.	8 hrs.	8 hrs.	8 hrs.
	50%	310 „	450 mins.	24 hrs.	22 hrs.	22 hrs.
	25%	480 „	11 hrs.	24 hrs.	24 hrs.	24 hrs.

Similarly, when extractions of enzyme powder were made int he same ratio in water, potato, carrot, swede and turnip juices, the times for maceration of the resulting preparations were 35 mins., 12 hrs., 150 mins., 130 mins., and 130 mins. respectively.

The following conclusions emerge from the above data :—(i) Plant juices, in general, have a retarding effect on the activity of the enzyme as shown by Brown (1915). (ii) The endo-enzyme of *Botrytis cinerea* is particularly affected by the raw juice of potato. The activity of the enzyme extracted in raw juice of potato is also low. (iii) With *Pythium*

de baryanum, potato juice does not have a great retarding effect, but juices of carrot, swede and turnip have strong inhibitory influence. These observations confirmed the earlier work of Chona (1932) on the differential effect of potato and turnip juices on enzymes of *Botrytis cinerea* and *Pythium de baryanum* and offer a line of approach to an investigation of the differential behaviour of these fungi on these hosts.

Behaviour of the fungi on living potato tissue

Inoculation experiments were carried out on cylinders of potato var. King Edward with *Botrytis cinerea* and *Pythium de baryanum* to examine in detail the course of events leading to unsuccessful or successful attack as the case might be. The method of procedure was that explained before. With *B. cinerea*, the inoculum was either a spore-suspension or a piece of mycelium with *Pythium de baryanum* the latter only. After inoculation, the cylinders were examined at 24 hours intervals for three successive days. The observations were made mainly on freehand sections of the fresh material which were stained with cotton blue. The effect is to stain the spores and mycelium blue against the colourless background of the cells. Scharlarch R. or Sudan III was used for the test of suberin. For detailed studies, the material was fixed in Craft A and B, and embedded in paraffin. The microtome sections (12–14μ thick) were stained in Haemotoxylin with Sudan III as counter stain (Dey, 1919). The results of the observations may be summarised as follows :—

With cylinders of potato inoculated with spores of *B. cinearea*, germination occurred freely within 24 hours and the germ tubes grew quite well. There was nothing to suggest any checking of the growth of the fungus at this stage. The fungal hyphae attacked the cells and a small amount of rotting was produced. Usually only a few layers of cells were involved; but after about 3 days, the presence of suberin in the walls of cells underlying the rotted tissue could be demonstrated. Similar suberisation was seen when mycelial inocula were used. It may be that the cork layer so formed constituted a mechanical barrier which led to stoppage of invasion, but it is equally plausible that invasion had been stopped by other factors and that cork-formation was the normal response of the healthy underlying cells to a wound stimulus.

With *Pythium de baryanum*, a progressive and gradual rotting occurred. The rotting was rapid to start with and no suberised layers were found to be present.. The main conclusion to be drawn from these observations is that germination of the spores is normal and that the factor which confers resistance comes into play after germination has taken place.

Behaviour of the enzymes on living potato tissue

Fernando and Stevenson (1952) have suggested that the enzyme of *Botrytis cinerea*, when placed on potato tissue, is unable to produce much rotting because it is rapidly absorbed by the cells of the potato. A series of experiments was carried out in which attention was paid to the rapidity with which a given small quantity of enzymic solution was absorbed into the tissue, in order to see whether there was any relationship between the speed of absorption and the amount of rotting produced.

The cylinders (1.8 cm. in diameter and 3 cm. in length) were cut from the tubers of var. King Edward and a small cavity (0.5 cm. in diam. × 1 cm. deep) made on the top of each cylinder. After thorough washing, small measured quantities (0.2 cc.) of enzymic solutions were placed in the cavities and the material was then incubated in moist dishes at 20°C. The results are assembled in Table 29 which gives the particulars of the various enzymes and enzyme mixtures (equal volumes) used. The figures represent the average of ten replicates.

Table 29. *Behaviour of various enzyme preparations of* B. cinerea *and* P. de baryanum *on potato tissue*

Enzyme	Time for maceration (in mins.)	Amt. (gm) of rotting of cylinders	Time for Absorption of 0.2 cc. of Enzyme Solution (hrs.)
1st Set			
1. *Botrytis*, endo. in water	25	0.066± 0.006	18
2. *Botrytis*, endo. in water deact	—	—	16
3. *Bot.*, exo. in Turnip Extract	50	0.057± 0.007	24
4. *Bot.*, exo. in Turnip Extract deact.	—	—	24
5. *Bot.*, endo. (water) *Bot.* exo. (T.E.)	35	0.065± 0.007	20
6. *Bot.*, endo. (water) *Bot.* exo. (T.E.) dact.	—	—	20
7. *Pythium*, exo. in Synthetic Soln.	25	0.28± 0.002	20
8. *Pyth.*, exo. in Synth. Soln. deact.	—	—	16
9. *Bot.*, exo (T.E.) *Pyth.* (exo.) in S.S.	35	0.13± 0.01	24
10. *Bot.* (exo.) (T.E.) *Pyth.* (exo.) in S.S. deact.	—	—	24
11. *Bot.* (endo.) (water). *Pyth* (exo.) in S.S.	25	0.143± 0.023	16
12. As above 11—deact.	—	—	16
13. *Bot.*, endo. in 7.5% Sucrose Soln.	75	0.07± 0.003	Not absorbed
14. *Bot.*, endo. in 15% Sucrose Soln.	105	0.07± 0.007	Not absorbed
15. *Pythium* exo. from Cylinders of Potato	270	0.05± 0.005	24
16. As 15 above, deact.	—	—	24
17. *Bot.* endo. (water) *Pyth.* (exo.)	150	0.09± 0.008	20
18. As 17 above, deact.	—	—	20

Table 30. *Progress of attack on potato tissue by various enzyme preparation of* B. cinerea *and* P. de baryanum

Enzyme	Time for maceration (mins.)	Amount of rotting of cylinders after 24 hours	48 hours	72 hours	Time for absorption of the enzyme solution (hrs.)
1st Set. (0.1 cc. of enzyme used)					
1. *Botrytis* endo.—(in water)	35	0.069± 0.003	0.070± 0.003	0.075± 0.009	16
2. *Botrytis* exo.—(in Turnip Extract)	45	0.069± 0.003	0.71± 0.009	0.079± 0.009	24
3. *Pythium* exo.—(in Synthetic solution)	45	0.213± 0.01	0.288± 0.014	0.352± 0.014	16
4. *Botriytis* endo.—(water) *Bot.* exo.	40	0.072± 0.004	0.081± 0.01	0.90± 0.007	24
5. *Botrytis* endo.—(water) *Pyth.* exo.	40	0.135± 0.012	0.180± 0.006	0.225± 0.012	16
6. *Botrytis* exo.—(T.E.) *Pythium* exo.	45	0.126± 0.012	0.187± 0.012	0.272± 0.011	24
2nd Set. (0.2 cc.) of enzyme used)					
7. *Botrytis* endo.—(in water)	30	0.107± 0.007	0.114± 0.09	0.128± 0.005	19
8. *Botrytis* exo.—(in Synthetic solution)	25	0.125± 0.008	0.132± 0.013	0.139± 0.012	20
9. *Pythium* exo.—(from inoc. cylinders of potato)	210	0.087± 0.01	0.112± 0.013	0.200± 0.018	24
10. *Botrytis* endo.—(water) *Botrytis* exo.	25	0.130± 0.012	0.138± 0.011	0.148± 0.016	20
11. *Botrytis* endo.—(water) *Pythium* exo.	45	0.087± 0.01	0.162± 0.013	0.208± 0.018	24
12. *Botrytis* exo.—(Syn Soln.) *Pythium* exo.	45	0.089± 0.01	0.156± 0.014	0.222± 0.027	24

The conclusions drawn from the above Table are as follows:—(i) *Botrytis* endo-enzyme when prepared in water (No. 1,) is absorbed in about 16–18 hours and produces no more than about 0.06 gm. of rotted tissue even when in high concentration (i.e., with maceration time about 25–30 minutes). (ii) *Pythium* enzyme of the same activity in maceration test (No. 7) in absorbed in much the same time but gives much increased rotting (0.28 g.). (iii) *Botrytis* enzyme of high activity but with osmotic contents present (No. 13, 14) is not absorbed, but yet gives little rotting (0.07 gm.). (iv) With preparations of low activity in the maceration test a comparatively greater amount of rotting is caused by *Pythium* enzyme than by *Botrytis* enzyme. (v) Enzyme mixtures give intermediate data as compared with each constituent. (vi) The rate of absorption of deactivated is the same as that of active enzyme. It appears, therefore, that the source of the enzyme i.e., whether from *Botrytis* or *Pythium* combined with its intrinsic activity as measured by the maceration test determines the amount of rotting which takes place. The time required for absorption by the tissue is not obviously of any significance.

The progress of attack with various enzymic preparations, was further examined in the experiments recorded in Table 30 and the following general conclusions may be drawn :—(i) With the enzyme of *Pythium de baryanum*, the rotting of potato cylinders is progressive with increasing time. This is shown clearly in cases of both stronger (No. 3) and weaker (No. 9) preparation. (ii) With the enzyme of *Botrytis cinerea*, both exo.- and endo.- the same tendency is evident, but in no case do the increases come up to the level of significance. This is more marked when a larger quantity is used (Nos. 7 and 8) than with a small quantity (Nos. 1 and 2). (iii) The mixtures, as before, behave intermediately between the two components.

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TWO DECAY FUNGI ON CONIFERS IN THE HIMALAYAS

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(With Plate I and 2 Text-figures)

Abstract

Lentinus lepideus and *Poria ferruginosa* causing brown cuboidal rot and white pocket rot respectively on coniferous wood in the Himalayas are recorded new in India. The two fungi have been described and their cultural characters studied.

The paper gives an account of two fungi, *Lentinus lepideus* and *Poria ferruginosa*, which principally cause decays on conifers in the Himalayas. Both the fungi are widely distributed in Europe and North America where they are of considerable economic importance. *L. lepideus* is tolerant to a comparatively light concentration of creosote (Cartwright and Findlay, 1946) and the fungus is commonly associated with the decay of timber used in service. It is not usually a parasite and is not recorded on hardwoods. *P. ferruginosa* is known abroad commonly as a decay fungus both on hardwoods and conifers. Both the fungi are recorded for the first time in India.

1. *LENTINUS LEPIDEUS* Fr.

Occurrence

Lentinus lepideus occurs commonly in the temperate regions of the western Himalayas at altitudes between 6,000—8,000 ft. in Kulu division, the Punjab, and Chakrata division, Uttar Pradesh. It occurs usually as a saprophyte on fallen logs of spruce (*Picea morinda*) and blue pine (*Pinus excelsa*) though on rare occasions it has also been recorded to grow parasitically on partly exposed healthy roots of living deodar (Pl. I, fig. 1) and spruce.

Sporophore

Fruit-body. Fleshy, tough and white when fresh, drying hard and brown. *Stalk.* Central, solid, white or brown with age, volva and annulus not found, about 2-9 cm. long, 0.5-1 cm. wide. *Pileus.* About 2-7 cm. in diameter. *Upper surface.* White when fresh, drying brown, with closely appressed brown scales (Pl. I, figs. 1 and 2), reticulately wrinkled, edge incurved. *Flesh.* Fleshy to corky, white to light buff*, about 0.7-1 cm. thick; hyphae hyaline, mostly thick-walled, with wide or narrow lumen (text-fig. 1, b), rarely thin-walled, sparsely branched, with simple septa and clamp connections (text-fig. 1, a), about 2–9μ broad. *Gills.* White when fresh, turning brown on drying, crowded (Pl. I, fig. 2), decurrent, edge serrate. *Basidia.* Elongated clavate (text-fig. 1, c), up to 25μ long, about

* Ridgeway (1912).

4.5—5.7μ broad; sterigmata 4 in each basidium, upto 3μ long. *Basidiospores.* Distinctly yellow in appearance when fresh, hyaline in old herbarium specimens, sub-cylindric or elliptical, thin-walled, with a short apiculus (text-fig. 1,d), dimension about 7—10 (12)×2.7—3.7 (4.1)μ.

As stated by Smith (1949), the species is variable in its character. All trace of veil, said to be present in young fruit-bodies, is lost at maturity. Cystidia are said to occur in the hymenium (Smith, 1949) but in our specimens, there are no structures distinctive from the basidia to be regarded as cystidia. A sporophore of the fungus collected at Madison, Wisconsin, U.S.A. and deposited in this herbarium, also, did not reveal the presence of cystidia. Basidiospores are said to be hyaline. In our specimens, they are yellow when a fresh discharge of spores is collected in the field, while in specimens kept dry in the herbarium for about two months, spores are hyaline. This is due, evidently, to fading out of the colour.

Specimens examined

On wood of *Pinus excelsa*, Bashahr, Himachal Pradesh, Oct., 1941, 5798;* on wood of *P. exelsa*, Konain, Chakrata div., Uttar Pradesh, June, 1947, 4803; on roots of living *Cedrus deodara*, Kasol, Kulu div., the Punjab, June 1955, 6542; on wood of *P. excelsa*, Kasol, June, 1955, 6539, and 6514; on roots of a living *P. morinda*, Pulga, Kulu div., the Punjab, June, 18, 1955, 6543.

Rot

The fungus causes a brown cuboidal rot in wood. The decayed wood becomes powdery under pressure of the thumb. White mycelial felts may be present on the surface of decayed wood and also within the cracks.

Cultural characters

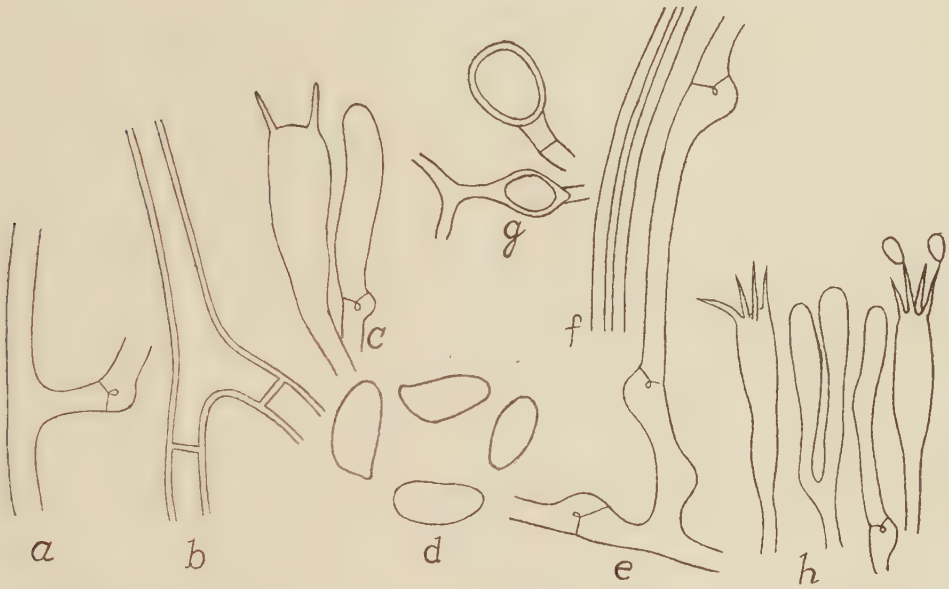
Growth Characters : Growth (radial) on *malt agar* 1.5 cm. in 7 days at 25°C. in darkness; mat thin, appressed; white when young, in 3 weeks becoming light buff which later deepen to warm buff; colour of the medium darkened; on gallic and tannic acid media diffusion zones present or absent, growth nil to 1 mm. in 7 days on gallic acid, nil on tannic acid.

Davidson, Campbell and Blaisdell (1938) also reported that *L. lepideus* in agar containing gallic or tannic acids sometimes formed diffusion zones and at other times the diffusion zones were absent.

Hyphal Characters : *Aerial mycelium*—(a) hyphae hyaline thin-walled, branched, with simple septa and clamp connections (text-fig. 1, e), about 1-4.4μ broad; (b) fibre-hyphae occur in old cultures, hyaline, but sometimes brown when very old, thick-walled, sparsely branched, distantly septate (text-fig. 1, f), about 1-2.8μ broad; (c) chlamydospores

* Numbers in italics indicate herbarium numbers of the specimens kept in Mycology branch, F.R.I., Dehra Dun.

numerous, globose to ovoid, hyaline, sometimes with light brown contents, thick-walled, apical or intercalary (text-fig. 1,g), dimension about 9-18 $\times$6.6-13μ. *Submerged myclium*—(a) thin-walled hyphae and (b) chlamydospores as in the aerial mycelium; (c) crystals needle-like and hexagonal.



Text-fig. 1. a, Thin-walled context hyphae with clamp connection ; b, thick-walled context hypha ; c, basidium ; d, basidiospores ; e, hypha in culture with clamp ; g, chlamydospores ; h, part of hymenium from fruit-body in culture showing basidia ($\times$1250)

Fruit-body develops readily in some cultures (Pl. I, fig. 4), completely lacking in some isolates, while in others pileus usually fails to develop (Pl. I, fig. 3) and the stalk branches in a coralloid manner. *Basidia.* Cylindric to narrow clavate (text-fig. 1, h), dimension about 8-9$\times$3μ, with 4 slender sterigmata on each, upto 4.5μ long. *Basidiospores.* Hyaline, elliptical, dimension about 7-8$\times$3-3.5μ.

2. *PORIA FERRUGINOSA* (Schard. ex Fr.) Karst.

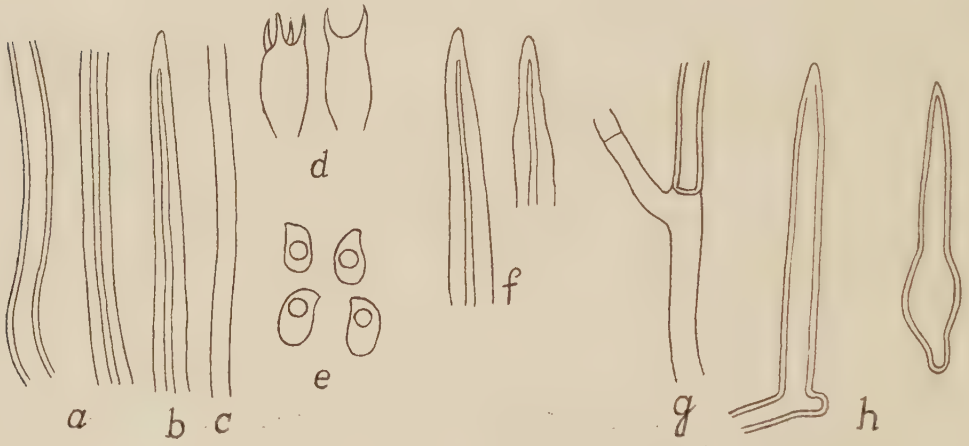
Occurrence

Poria ferruginosa has been collected, though not commonly, from the western Himalayas in the Chakrata and Kulu divisions, at altitudes of 6,000—8,000 ft. The fungus has been found growing only on coniferous wood.

Sporophore

Fruit-body. Annual, broadly effused, inseparable from the wood, corky, margin sayal brown, narrow, thin, sterile. *Subiculum.* Brown,

0.15-0.2 mm. thick ; hyphae brown, thick-walled with wide or narrow lumen, unbranched, apparently aseptate (text-fig. 2,a), about 2.1—3.8 μ broad, dark brown setae-hyphae with pointed ends present (text-fig. 2,b), about 6—10 μ broad, frequent; tramal hyphae similar to hyphae of the subiculum, hyaline, thin-walled, apparently unbranched, indistinctly septate sayal brown or snuff brown; dull; pore-tubes 1—2 mm. long, pore-months circular to slightly angular, irpiciform when growing on vertical surfaces (Pl. I, fig. 5), 3—5 per mm., edges entire. *Basidia.* Clavate (text-fig. 2,d), about 4.3—5.1μ broad. *Basidiospores.* Hyaline, thin-walled, smooth, oblong, apiculate, with 1-guttulate (text-fig. 2,e), dimension about 5.5—6.1×3.0—3.8μ. *Setae.* Dark brown, subulate, acuminate (text-fig. 2,f), abundant, dimension about 48—68 (102)×6.8—9.5μ.



Text-fig. 2. a, Thick-walled hyphae of the subiculum ; b, setae like hypha from the subiculum ; c, thin-walled tramal hypha ; d, basidia ; e, basidiospores ; f, setae in the hymenium ; g, hypha in culture hypha ; h, setae in culture (Figs. a, c-e, g, × 1250 ; b, & h, × 500).

Specimens examined

On wood of *Pinus excelsa* in buildings, Deoban, Chakrata div., Uttar Pradesh, July, 1945, 4040(*a*); on *Cedrus deodara* beams, Kasol, Kulu div., the Punjab, June, 1955, 6536 and 6537.

The fungus closely resembles *Poria viticola* Schw. (= *Trametes tenuis* Karst.) from which the present species can be distinguished from the character of the spores (Overholts, 1922). Further *P. ferruginosa* produces abundant setae in culture while *Trametes tenuis* Karst. as described by Nobels (1948) has no setae in culture. The affinities of the fungus are also with *Poria ferrea* (Pers.) Bourd. & Galz. and *P. ferrugineo-fusca* Karst. from which *P. ferruginosa* is distinguished by the character of basidiospores.

Rot

The fungus causes a white spongy rot,

Cultural characters

Growth Characters : Growth (radial) on *malt agar* about 0.5 cm. in 7 days at 25°C in darkness; mat dirty white, loosely woolly on the inoculum, short cottony around it, becoming thin felty and developing buckthorn brown and ochraceous tawny with age which later deepen to russet in patches; reverse developing same shades belov coloured areas of the mat.

The texture and the colour of the mat are dependant on temperature. At lower temperatures (18-23°C) there is satisfactory development of aerial mycelium, the mat is white for most part and the colour development is delayed and less intense. At higher temperatures (26—30°C) the aerial mycelium is scanty, colours appear quickly and the shades are more intense.

Hyphal Characters : *Aerial mycelium*—(a) hyphae hyaline, thin-walled, branched, septate (simple), becoming pale brown and slightly thick-walled (text-fig. 2,g), about 2.2—4.4μ broad; (b) setate dark brown, subulate (text-fig. 2 h), abundant, 61—110$\times$5—10.2μ.

Fruit-body sometimes develops in test tubes in 5—6 week old cultures, consisting of irregularly poroid raised surfaces, vinaceous buff, avellaneous to light brownish drab in colour. *Basidia.* Hyaline, broad clavate,upto 5.1μ broad, with 4 long sterigmata. *Basidiospores.* Hyaline, thin-walled, smooth, oblong. apiculate, with 1-guttulate, dimension about 4.8—6.0$\times$3.0—3.6μ.

Acknowledgment

Our thanks are due to Miss E. M. Wakefield for kindly indentifying the sporophores of *L. lepideus* and to Dr. K. Bagchee for taking the photographs 3 and 4.

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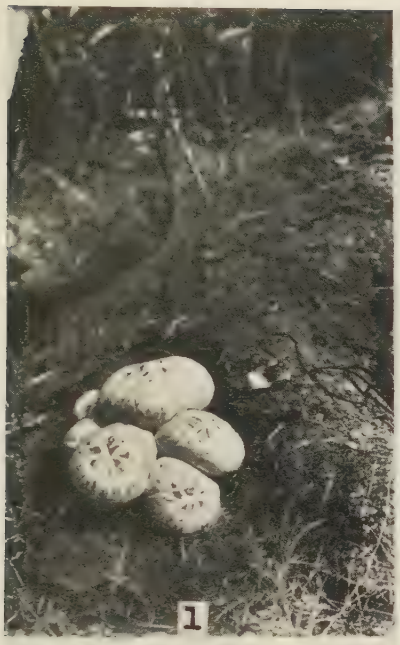
Explanation of Plate I

Fig. 1. *Lentinus lepideus*, sporophores on exposed roots of a living deodar (×1/5).
Fig. 2. *Lentinus lepideus*, sporophores showing hymenial surface (×3|4).
Fig. 3. *Lentinus lepideus*, immature sporophore (with stalks only) growing on malt agar in Kolle flasks (×2/5).
Fig. 4. *Lentinus lepideus*, typical sporophore on malt agar in Kolle flasks (×1/2).
Fig. 5. *Poria ferruginosa*, sporophores showing hymenial surface (×1).



Two Decay Fungi on Conifers Bakshi, Puri & Singh

MONSOON DAY FORAYS, 1955.

The usefulness of Mycological Forays has been emphasised in the Foreword of the President of our Society. From the beginning, good response in this regard was forthcoming from members as well as from visitors who are welcome in our Forays. Institutions and other sister societies, also extended their hand in making these Forays attractive and successful, and I would like to thank them all, particularly, Dr. B. C. Kundu, Director, Jute Agricultural Research Institute for giving our members a grand welcome.

Day Forays organised by the Mycological Society were held as follows : *10 July*, Indian Botanic Garden, near the Great Banyan tree, 1st area; *17 July*, Indian Botanic Garden along the Ganges side, 2nd area; *31 July*, Thakurpukur; *7 August*, Jute Agricultural Research Institute, Barrackpur; *21 August*, Baruipur. All Forays were held under the leadership of Dr. S. N. Banerjee, the Vice-President of the Society with an average of over 20 members particepating.

Days on which Forays were held had been mainly cloudy with intermittant showers. Weather was, however, bright and sunny on the *31 July* and on preceeding days. An outstanding feature of the Forays at Indian Botanic Garden on the *10 July* was the formation of a 'fairy ring' of *Lycoperdon* sp. on a grassy lawn.

List of Specimens Collected during the Forays

Introduction.—In this list, the locality in which a specimen had been found is indicated by letters in parantheses : Indian Botanic Garden (I); Thakurpukur (T); Barrackpur (N); Baruipur (B).

Agaricus campestris L., on humus soil in open field, (T); *Auricularia auricula-Judae* (L.) Schroet., on dead wood, (B); *A. rosea* Burt., on dead wood, (I); *Clavaria angulispora* Pat., on humus, (N); *Collybia velutipes* (Curt.) Fr., on decaying wood and on stumps, (I); *Coprinus comatus* Fr., on decaying leaves and twigs, (I & N); *C. micaceus* (Bull.) Fr., on humus soil, (I); *C. fibriatus* B. & Br., on dung, (I); *Craterellus cornucopioides* (L.) Pers., in clumps on Bamboo and on humus, (I); *Cyathus intermedius* (Mont.). Tul., on decaying twings and leaves, (I); *Daldinia concentrica* (Bolt.) Ces. & de Not., on dead wood, (N); *Entoloma microcarpum* (B. & Br.) Sacc., on old termite nests or on soil, (I); *Flammula dilepis* B. & Br., on stumps of Bamboo and Cocoanut, (T); *Fomes durissimus* Lloyd, on trunks on living *Swietenia* and *Casuarina* trees, (I); *F. senex* Nees. & Mont., on living trunk of *Swietenia*, (I); *Ganoderma colossum* (Fr.) Bres., at the base of the trunk of Banyan tree, (I); *G. lucidum* (Leyss.) Karst., at the base of the living *Swietenia* and *Casuarina* trees, (I); *G. applanatum* (Pers.) Pat., on dead Mango wood, (I); *Grammothele effuso-reflexa* Banerjee, on logs of *Shroea rolusta*, (T); *Guepinia spathularia* (Schw.) Fr., on dead stumps of Bamboo, (B); *Hexagonia apiaria* Pers., on dead twig of Mango, (N); *H. discopoda* Pat. & Har., on dead twigs of Mango, (N); *Irpex flavus* Kl., on dead wood of Mango, (T); *Lentinus praerigidus* Berk., on dead log of *Shorea robusta*, (N); *L. sajor-caju* Fr., on dead wood, (I), *L. subnudus* Berk., on dead wood of *Shorea robusta*, (I); *Lenzites repanda* (Mont.) Fr., on stamps of Bamboo, (T); *L. striata* Swartz., on dead wood, (B); *Marasmius campanella* Holterm., on living trunks of *Lagerstroemia speciosa*, (B); *Merulius similis* B. & Br., on stumps of Bamboo, (B); *Panaeolus campanulatus* (L). Fr., on dung and in pastures, (I); *Polyporus agariceus* Berk., on log of *Shroea robusta*, (T); *P. anthelminticus* Berk., on humus soil, (B); *P. brumalis* (Pers.) Fr., on dead wood, (N); *P. durus*

Jungh., on stumps of Bamboo, (T); *P. friabilis* Bose., on stumps of Bamboo, (T); *P. grammocephalas* Berk., on dead trunk of *Spondias* wood, (N); *P. luzonensis* Murr., on dead trunk of Banyan, (N); *P. ochroleucus* Berk., on posts of *Shorea robusta*, (T); *P. zonalis* Berk., on post of *Shorea robusta*, (I); *Polystictus hirsutus* Fr., on logs of *Shorea robusta*, (T & B); *P. leoninus* Klotz., on branch of Mango tree, (N); *P. sanguineus* (L.) Mey., on logs of *Shorea robusta*, (N); *P. steinheilianus* Berk. & Lev., on logs of *Shorea robusta*, (I); *Poria diversispora* B. & Br., on stump of Bamboo and on log of *Shorea robusta*, (I); *Psathyrella disseminata* (Pers.) Fr., on decaying wood, specially much decayed ones and on ground, (I); *Stereum elegans* Fr., in rosettes, usually on ground near the base of a tree, sometimes at the junction of the log and the soil, (I); *S. fuscum* (Schard.) Quel., on dead trunks and on logs of *Shorea robusta* (I & N); *S. nitidulum* Berk., on dead stumps and half burried wood, (I); *S. percome* B. & Br., on dead branches and on logs of *Shorea robusta*, (T & N); *S. petalodes* Berk., is rosettes, usually on ground near the base of living *Grevillea robusta* tree, (I); *Trametes badia* (Berk.) Cooke, on logs of Banyan, (N); *T. cingulata* Berk., on log of *Shorea robusta* and bark of living Cocoanut palm, (I & N); *T. floccosus* Bers., on bark and trunk of *Saraca indica*, (I & B); *T. lactinea* Berk., on dead wood of Cocoanut palm, (B & N); *T. meyeni* Klotz., on dead wood of Banyan, (I); *T. persoonii* Fr., on dead wood of Banyan and *Shorea robusta*, (I, N & T); *T. versatilis* Berk., on logs of Cocoanut palm and *Shorea robusta*, (B & T); *Tricholoma gigenteum* Massee, on trunk of *Swietinia* near the ground, (I).

In addition to species listed above, 39 species of Agarics distributed among 20 different genera were, also, collected. These species are still under investigation.

Hashi Basu,

Foray Secretary.

Jute Agricultural Research Institute

Thakurpukur

Indian Botanic Garden

Monsoon Day Forays, 1955 Hashi Basu

EXTRACTS
from
RULES AND REGULATIONS
of
MYCOLOGICAL SOCIETY

Name and Objects

The Society shall be called "Mycological Society." The aims and objects of the Society shall be :—(a) to promote the cause of Mycology and allied subjects like Microbiology, Soil Bacteriology, Plant Pathology, Fungous Physiology and so on; (b) to hold periodical meetings in Calcutta as well as in other parts of India with a view to diffuse knowledge in the subjects referred to above, among the public and facilitate mutual intercourse among members; (c) to encourage original investigations; (d) to arrange periodical excursions to different parts of India for the study of Fungal and allied flora; (e) to co-operate with other scientific bodies for purposes of common interest; (f) to publish Transactions, Journals, Memoirs and other matters of the Society as may be desirable; (g) to perform all acts that may assist in, conduce to or be necessary for the fulfilment of the aims and objects of the Society.

Membership

All persons, irrespective of religion and sex, interested in any phase of Mycology and allied subjects, as referred to above, pure or applied, shall be eligible for membership.

The Society shall consist of the following classes of members:—(a) Ordinary members, (b) Honorary members, (c) Life members and (d) Associate members.

Members shall be admitted to the Society on the approval of the Executive Council after being duly proposed and seconded by Ordinary members in the prescribed form (Appendix).

Ordinary Members shall be entitled to all the privileges of the Society and shall receive, free of charge, all publications of the Society, except such special publications which that Executive Council may decide to issue to members on payment of the cost of publication. Annual subscription for Ordinary Members shall be Rs. 15/- only. Associate Members shall have to be certified as *bonafide* students to the satisfaction of the Council. They shall form a separate Constituency with a limited voting right and shall elect one representative to the Executive Council from this constituency. They shall get the publications of the Society at reduced

rates to be determined by the Executive Council. Annual subscription for Associate Members shall be Rs. 5/- only.

Any eligible person paying at a time Rs. 200/- only shall be Life Member. A Life Member shall enjoy all the privileges of the Society as an Ordinary Member.

Any person or association or corporate body or the University, approved by the Executive Council, shall, on payment of Rs. 500/- only or upwards, be regarded as a DONOR, and will enjoy the privileges of a Life Member.

Subscriptions for Ordinary and Associate Members shall be due on the *First* day of January, every year. Members whose subscriptions are in arrears for more than one year, shall be exluded from privileges of membership.

ADMINISTRATION

The administration of the Society shall vest in an "Executive Council", hereinunder referred to as the *Council*.

The Council shall consist of :

(a) One President,
(b) Two Vice-Presidents,
(c) One Secretary,
(d) One Joint Secretary,
(e) One Treasurer,
(f) One Editor of Publications, elected by the Council.
(g) Eight other members, of whom not more than one shall be elected from any institution, including one woman member,
(h) One Associate Member, who shall be elected from Associate Members' Constituency.
(i) One Foray Secretary, elected by the Council

The members of the Council shall be in their office in honorary capacity.

Members of the Council, excepting the Treasurer, the Secretary, the Joint Secretary and the Editor of Publications, shall hold office for one year. The Treasurer, the Secretary, the Joint Secretary and the Editor of Publications shall hold office for three years. All elections excepting these shall take place at the Annual General Meeting of the Society to be held within March every year or on the date fixed by the Council, interim vacancies being filled up by the Council. The Council shall continue in office, notwithstanding that their office may have expired, until their successors have been duly appointed and have taken over office. All retiring members of the Council shall be eligible for re-election. The first Council formed shall function for a period of three years.

MYCOLOGICAL SOCIETY

LIST OF FOUNDATION MEMBERS

Life Members

1. Banerjee, Dr. Sachindranath
   33/8A, Sashi Bhusan De St.,
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